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THE FOLIOSE AND FRUTICOSE LICHENS OF COSTA RICA. I

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While there exists much literature on various groups of lichens from various parts of tropical America, at present I know of no recent work covering the whole area or that of any constituent country whereby the traveler may identify the lichens he meets, or the student in an herbarium of the temperate zone may readily determine any considerable portion of the miscellaneous material sent in by collectors. It is hoped that this work may furnish such a manual, although no one realizes better than I the huge amount of monographic work necessary before a book approaching completeness and accuracy can be published.

I first came under the spell of the rich flora of Costa Rica during a two-weeks visit in the summer of 1925 and at that time formed the resolution to spend my first sabbatical year in that country in the study of its fungus and lichen flora. During the subsequent years much material in addition to my own collections came to me for identification. Plans were matured and in early September, 1929, my family and I landed in Costa Rica and made our headquarters in San José, the capital of the country. In the latter part of September I was joined by Mr. W. Stephen Thomas, a student at Harvard, who accompanied me on most of the collecting trips until the middle of March. Mrs. Dodge also spent some time in the field and much is owed to her keen observation and helpful care of the collections. Even the small daughter, at the age of four, added a number of specimens

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not otherwise easily obtained, through her skill in climbing guayabos (*Psidium guajave* L.) which were too small and too brittle to be climbed by an adult. We left Costa Rica in June, 1930, and after a brief stay at the Farlow Herbarium to sort over material and give tentative determinations I took the more puzzling specimens to Europe where the months from August to December, 1930, were spent in the larger herbaria.

Very many persons and institutions have contributed to the success of this undertaking both at home and abroad, and to all I tender my most hearty thanks in grateful acknowledgment of their many kindnesses. For financial assistance, I am grateful to the John Simon Guggenheim Memorial Foundation which appointed me as a Fellow to Costa Rica for the year 1929–30 and to Europe from October to December, 1930; to the President and Fellows of Harvard College and to the Farlow Herbarium, for equipment, incidental expenses, and for an assistant in the field; to my colleague, Prof. Greenman, and former colleagues, Professors Ames, Barbour, and Thaxter, and to Mr. Paul C. Standley of the Field Museum, for excellent advice in planning my trip and for helpful letters of introduction. Members of the staff of the United Fruit Company were also very helpful both in planning the trip and placing the facilities of that great company at my disposal in Costa Rica, especially Dr. J. R. Johnston of the Boston office, and Messrs. Kress, Fuller, Crawford, Stübbe, and George Catt, the director of its Botanical Garden and Experiment Station at Siquirres, who accompanied me on several trips in that vicinity.

Among Costa Ricans, I am deeply indebted to the following for advice and hospitality, without which I should have been unable to cover so much territory so thoroughly in so little time: Bernado Yglesias Rodriguez, the Director of the Escuela Nacional de Agricultura, who placed the facilities of the botanical laboratory at my disposal and enabled me to spend three very profitable weeks at the ancestral Yglesias finca, Guayabillos, on the upper slopes of the western face of Irazú; to Anastasio Alfaro, formerly director of the Museo Nacional (and later to his successor, the late J. Fidel Tristan), who placed the facilities of the library and herbarium (Wercklé) of the institution at my dis-

posal and suggested the plan of my trip to Guanacaste, as well as contributing several specimens; to Dr. Alberto M. Brenes, the botanist at the Museo Nacional, who collected extensively for me in the vicinity of San Ramón, an interesting region which I did not have an opportunity to visit personally; to Ricardo Chavarría Flores, formerly city engineer of Cartago, who enabled me to collect extensively at his finca in the vicinity of Santiago de Cartago and the Rio Birrís; Juvenal Valerio Rodriguez, of the Instituto de Alajuela, who planned trips in the vicinity of Alajuela and to the Cerros de Zurquí; Otón Jiménez Luthmer, of the Botica Oriental, a well-known amateur botanist, who gave generously of his time in suggesting trips and making plans, although his business duties prevented his accompanying me in the field except for a short time on our trip to Guanacaste; to Ferdinand Nevermann, the coleopterist, for numerous pleasant collecting trips, including a three-weeks stay at fincas of which he is manager in Limón Province, and for much helpful information in connection with my large insect collections; to Ruben Torres Rojas, of Cartago, for specimens, although I did not have the pleasure of a personal contact with him during my short visits to Cartago; and to Charles H. Lankester, for helpful suggestions. Besides these who are more or less professionally interested in natural history, the following were generous in their hospitality and enabled me to visit regions which would have been otherwise quite inaccessible to the traveler: Gonzalo Volio and his son Carlos, Antonio Sobrado and his brothers, Carlos Collado, Fernando Castro, José Castro Araya, Antonio Gutiérrez, José Luis Sancho, Carlos Piedra and son, Alexander Ross and sons, Robert Hanckel, and H. J. Marks.

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at Genève; and the Laboratoire de Cryptogamie at Paris, as well as the private herbarium of M. le docteur M. Bouly de Lesdain of Dunkerque. I am also indebted to the following lichenologists, whom I met during my trip, for helpful suggestions and criticism: Miss A. L. Smith, Bernt Lynge, Einar DuRietz, the late Karl Schulz-Korth, J. Motyka, and Ove Høeg. Since my return to America I have had access to or seen specimens from the following herbaria, for which I gratefully acknowledge my indebtedness to the curators: Farlow Herbarium of Harvard University, New York Botanical Garden, National Herbarium of the Smithsonian Institution, the Field Museum of Natural History, Chicago, the herbaria of the University of Michigan and of the Missouri Botanical Garden. I am also grateful to the Director of the Missouri Botanical Garden and to the Chancellor of Washington University for a leave of absence after the appointment to the staffs of those institutions, in order that I might complete certain studies in the Farlow Herbarium of Harvard University before I left that institution. Finally I wish to thank any others through whose hospitality and assistance this work has been made possible.

Geography

Topographically Costa Rica may be divided into four main phytogeographic regions: the Atlantic Coastal Plain, the Meseta Central, the Subalpine Region, and the Pacific Coastal Region, the latter less well known and less homogeneous.

The Atlantic Coastal Plain is characterized by its gentle slope from the sandy beaches up to about 100 m. or even higher in the poorly known Llanos de Santa Clara and in the Sarapiquí, San Carlos and Río Frío valleys. The beaches are interrupted only by shallow harbors at Cahuita, Limón, and Moin, the first being protected by a coral reef, the two latter being partially protected by the small island Uvita, now used as a quarantine station for Puerto Limón. Several large rivers rising in the highlands cross this plain and during their flood states overflow, forming spectacular anastomosing channels which leave small pools in the dry season. Most of the river mouths are closed by shifting bars which hinder or prevent the use of the rivers for

LAGO DE NICARAGUA
NICARAGUA
LLANOS DE SAN CARLOS
ALAJUELA
SAN JOSÉ
OCEANO PACIFICO

COCKAYNE, BOSTON.

shipping. Just back of the beach there is an almost continuous series of lagoons, which may be dredged and may be used for transport of the farm produce to the railroads and ports. These lagoons extend most of the way from San Juan del Norte to Limón.

The soil is quite fertile where drainage is sufficient and much of the forest has been cleared for agricultural purposes, in the Llanos de Sta. Clara and Limón. The rainfall is heavy and quite evenly distributed, although somewhat less during January, February, and March, the so-called dry season (verano). During most of the year the sun shines until about noon, then showers may be expected for either a short time or for the rest of the afternoon. The nights are usually clear and cool.

During the colonial era there was very little permanent settlement of this region although many attempts were made. Some cacao plantations were developed at Matina, and their product taken overland via Cartago to Honduras and Guatemala or quietly sold to the English pirates and smugglers who frequently entered the mouth of the Matina River for this purpose. With the advent of the Northern Railroad in 1870–1890 the banana industry spread rapidly over most of the available land from the Parismina to the Estrella River valleys and up the Sixaola from Bocas del Toro. When the banana wilt or Panama disease (*Fusarium cubense* E. F. Smith) and increasing soil exhaustion began to threaten this industry, coconuts, pineapples, and cacao were extensively planted in suitable localities. Coconuts were a prompt and complete failure owing to bud rot and other factors, although they still furnish enough for local consumption. Pineapple cultivation has recently been abandoned for economic reasons, although the product was much superior to that of Hawaii. Cacao cultivation has been only partially successful owing to high labor costs. Recently much land has been practically abandoned and is gradually returning to second-growth forests. While small plantations of forest trees, as *Hevea*, *Ochroma*, etc., are still in an experimental stage, they seem to be successful botanically but owing to the high cost of labor they are still doubtful economically. Maize is sometimes grown on the Llanos de Santa Clara.

The Atlantic Coastal Plain penetrates far inland in the Tortuguero, Sarapiquí, San Carlos and Río Frío systems, but since I have not had an opportunity to examine them personally and have seen very few specimens from them it is impossible to discuss them further. Before the railroad was built the Río Sarapiquí was the main route from San José to the Atlantic, and a few villages still remain. There are a few scattered settlements on the Río San Carlos and Río Frío, as may be seen from the relations of Thiel and Cespedes, but except for a few specimens by Pittier from the upper Sarapiquí Valley and from the vicinity of San Rafael on the R. San Carlos, I know of no lichen collections from this region.

The Atlantic Transition Zone from 100 to 900 m. is evident mostly in narrow river valleys, the only botanically well-known portions being the southeastern portion of the Llanos de Santa Clara around Guápiles, formerly an important farm of the United Fruit Company used for their earlier experimental plantings of forest trees, and the valley of the Reventazón.

In the valley of the Reventazón, tertiary sedimentary rocks are evident in the cañons and railway cuts. The slopes are very steep, due to the great erosion caused by the heavy rainfall which is about the same as that of the Coastal Plain. Bananas and coffee form the principal crops in the broader side valleys, the banana being comparatively short-lived but much used as a shade for the coffee. Since this development is comparatively recent, it still remains to be seen whether this is a permanent stage or a temporary phase in the agricultural development of the region. Formerly sugar cane was extensively cultivated in this region and considerable areas remain in Juan Viñas and Santiago de Cartago. Pineapples are grown for the domestic markets near Turrialba and to a less extent in some other places. Owing to the rapid erosion it seems doubtful whether much of the land which has been cleared on such steep slopes can remain permanently in cultivation. There are available a few collections from Angostura near Turrialba by Polakowsky and my own from the beautiful valley of the Río Pejivalle near the United Fruit Company's farm of that name, as well as the even more extensive ones from Santiago de Cartago and occasional specimens from

Juan Viñas (Naranjo) by Ørsted and scattered numbers by Pittier.

On the practically inaccessible slopes and in the valleys where transportation is too costly to make farms economically profitable yet, some forests are still to be found. These still resemble those of the Atlantic Coastal Plain with their abundant epiphytes, bromeliads being conspicuous, especially *Tillandsia usneoides*. There are many small areas among the hills where fogs keep the humidity much higher than in neighboring areas. These are quite evident by the abundance of the long pendulous species of *Usnea* which replace *Tillandsia usneoides* as a prominent feature of the landscape. Species of *Leptogium*, *Parmelia*, *Anaptychia*, and mosses are also very abundant. Cladonias begin to appear and replace the similarly colored moss of the lower elevations. In the top of the forest trees and in the isolated trees of the potrero (pasture), crustose species are abundant. In the lower levels of the forest, lichens are rarer and usually sterile, perhaps due to the small amount of light. Trees whose bark scales off rapidly, such as the guayabo (*Psidium guajave* L.), are rarely found to have lichens, except for a few fruticose species of *Ramalina* and *Usnea*. *Nephroma* was found in one of these *Usnea-Tillandsia* zones on an exposed "knife-edge" protruding into the cañon of the Reventazón near Santiago de Cartago.

The Meseta Central, occupying the huge central valley between the Cordillera Central and the Cordillera de Talamanca, consists of long gentle slopes between 1000 and 1700 m. cut by deep river gorges. The pass of Ochomogo and the Cerro de Carpintera divide this meseta into two portions, the eastern valley of Guarco, comprising the upper portion of the valley of the Reventazón and its tributaries, and the western valley of San José, comprising the upper part of the valley of the Río Grande de Tarcoles and its tributaries. The valley of Guarco is bounded on the north by the upper slopes of the volcanoes Irazú and Turrialba and on the south by the continental divide in the Cordillera de Talamanca. Most of the large tributaries enter from the south and are not explored fully from a geographical standpoint. I have seen lichen collections from the valley of the Río Navarro and the

lower part of the Orosí. The Río Grande is by far the largest and longest of the tributaries but its flora is wholly unknown to me. Coffee is the principal crop though considerable amounts of garden vegetables, especially chayote and yuca, are raised to supply not only the local markets but also the demands of the Atlantic Coastal Plain. Cartago, the colonial capital, is the only city but there are numerous small towns. The collections of C. Wercklé, "near Cartago 1200–1500 m.," in San José and Berlin, seem to be from the region about Aguacaliente and the lower slopes of the Cerro de Carpintera, judging from species represented.

The valley of San José is bounded on the north by the continental divide of the western portion of the Cordillera Central, the volcano Poás, and Barba with its lesser peaks, the Cerros de las Caricias, Las Lajas, and Zurquí, on the east by Irazú and the Cerro de Carpintera, on the south by the Cerros de Escasú and Piedra Blanca, on the west by the Cerros de Aguacate. In the valley of San José the slopes descend gently to about 650 m. This valley is much drier than the Guarco with a longer dry season from December to April and less rain in the other months. The winds are strong, especially in the dry season. Practically the whole region has been denuded of forests, partly in the interests of agriculture and partly owing to the fiendish glee of the inhabitants who deliberately set fires to the dry grass on windy days in order to watch it burn. By the end of the dry season practically the whole valley outside the cafetales and cane fields is black from the charred remains of grass fires.

The underlying rock of both valleys is largely volcanic, while the soil above is largely of aeolian origin from the volcanic dust which is still being regularly emitted from Irazú and occasionally from Poás. In some places fields of volcanic boulders exist in such profusion that they make agriculture as difficult as do the glacial boulders in parts of New England. The greater portion of both valleys is devoted to coffee growing, with sugar cane and upland rice in the lower levels. Formerly considerable excellent tobacco was grown but this crop has practically disappeared. The cities of Alajuela, Heredia, and San José, and many important towns are located in this valley.

Crustose lichens are abundant in the cafetales both on the coffee and its shade, but practically no attempt was made to collect them since the thalli are mostly young and sterile and we hesitated to injure the trees by removing portions of bark. The living fence posts, mostly species of *Erythrina* (poró), were utilized, however, since they are regularly and heavily pruned back. These yielded most of the crustose species from this area. Another fruitful source was the roadside bank. These banks are caused by erosion and by cutting down with spades to secure material to fill in holes in the road. The depth of a road in most places is proportional to the slope and to its age, a recently laid-out road having only slight banks while an old road often has vertical sides rising 15–30 feet above the wheel tracks. These banks have a wealth of lichens, bryophytes, and fungi during the rainy season (invierno) but usually dry out, except in occasional moist shady spots, during the dry season. Consequently most of the species are annuals and disappear quite completely during the dry season. These banks are even more conspicuous and important in the Subalpine Region.

The Subalpine Region probably includes most of the land in Costa Rica above 1700 m., but is known botanically only on the Cordillera Central and the northernmost mountains of the Cordillera de Talamanca as far as the Cerro de las Vueltas. Poás, Irazú, and Turrialba are largely cleared on their southern slopes and used for dairy farms. Barba is still largely wooded and inaccessible, but Standley and Maxon have explored the slopes of the lower peaks of the Cerros de las Caricias, las Lajas, and Zurquí. I also had the opportunity to spend two days on the lower slopes of Zurquí. Standley and J. Valerio secured an extensive series of lichens from Cerro de las Vueltas and neighboring peaks within reach of Santa María de Dota, in 1925–6. Various collectors have brought back small series of specimens from Poás. Standley in 1924 and I in 1929 collected on the south slope of Turrialba for a short period, and I spent about three weeks at Guayabillos on the west slope of Irazú, as well as climbing to the crater twice from the south side. Ever since the time of Ørsted many collectors have brought in a few specimens from Irazú. The alpine and subalpine areas of Irazú

are much less rich than the other mountains probably because of the constant dust. On my last ascent I did not find even a sterile thallus above 3000 m., while Ørsted reports lichens from the summit. Botanically this Subalpine Region is the most interesting, as it has a high percentage of Colombian and other South American species, as well as most of the endemic species.

The Pacific Coastal flora is too poorly known to classify into geographical divisions. I have been told by lumbermen that there are many interesting local distributions of the forest trees but these observations are too fragmentary for generalizations. For present purposes we may subdivide into the province of Guanacaste the peninsula of Nicoya, the great river valleys of the R. Naranjo, R. General, and R. Grande de Térraba or Diquís, and the Peninsula of Osa. Of these regions I have personally visited only the first and the last. The peninsula of Nicoya has not been explored at all as far as concerns lichens, while our knowledge of the great river valleys is due solely to two journeys by Pittier, Biolley, and Tonduz, in January and February, 1891, and by Tonduz from October 18, 1891, to April 17, 1892 (see p. 388).

The province of Guanacaste consists of a low coast range penetrated by the Bahías de Culebra and de Salinas, a broad valley of the R. Tempisque about 40 km. wide and 60 km. long, rising gently from sea level to about 200 m. and sloping again gently to the Lago de Nicaragua, with Liberia, the provincial capital, at its center, and the west slope of the Cordillera de Tilarán (or de Guanacaste) on its east. Ørsted crossed the plain from the Tempisque to the Bahía de Salinas and explored the region of R. Sapoa and the coast, but I have seen no lichens from this region. Pittier collected along the shore of the Bahía de Salinas and may have penetrated the low coast range. Otherwise it is unknown as far as lichens are concerned. The plain of the Tempisque is used for grazing with some sugar cane grown in the southwest part in the vicinity of El Paso. The underlying rock and soil in Liberia is white cascajo, a soft pumice laid down in water and hence somewhat stratified. Further south there are clay deposits. Rainfall is high during the rainy season but the dry season is hot and dusty, unofficial temperature in the

shade rising as high as 108°F. in the hottest part of the day and correspondingly cool at night. Wind velocities are high where the trade winds sweep through the passes between the volcanoes in the first half of the dry season, so that most of the perennial vegetation is that of the arid regions and may account for the similarity of the flora with that of Nicaragua and the north. Here grass fires are even more frequent in proportion to the population and more destructive, hence the region is seldom visited by botanists; in fact I have failed to find any collections from this region or any mention of it in botanical works. In 1930 I was able to spend about two weeks in the vicinity of Liberia.

The western slopes of the Cordillera de Tilarán are used for grazing, with small cafetales located in sheltered, moister areas. The vegetation does not appear much different from the plain of the Tempisque, especially on the exposed slopes, but in the sheltered ravines more delicate types survive. This region has not been visited by botanists to any great extent, although Standley and J. Valerio collected in the vicinity of Tilarán in 1926, and Valerio probably made studies of the local flora of Tilarán while he was stationed there as a teacher some years ago. Besides a week in Tilarán I spent about a week at 700 m. on the slopes of Santamaría, the next peak south of La Vieja, and a week at La Granadilla on the plateau between the R. de las Cañas and the R. San José which culminates at 960 m. in the Cerro de San José.

In Santamaría and Tilarán, even during the dry season, much of the time the weather was characterized by mists and high winds and much rain, while a short distance lower and to the west there was neither mist nor rain. However, the brief intervals of hot sun are sufficient to keep the xerophytic vegetation on the wind-swept prairie, while the sheltered ravines and shaded hillsides have mesophytic types. At Tilarán we crossed the continental divide into the headwaters of the Río Frío system with a change in vegetation even more striking than in the pass at Ochomogo in the Meseta Central.

The Cerro de San José rises sharply above the surrounding peaks to 960 m., somewhat higher than the near-by peaks of the

continental divide. It seems to be composed of stratified sedimentary rock sloping 40° S, 70° W. To the west rises the lower exploded crater of Cerro Pelado, while to the east lie the lower peaks which form the junction of the Cordillera de Tilarán and the Cordillera Central. Though the region is considerably drier than the same elevations on the Cordillera de Tilarán, yet it maintains a luxuriant forest vegetation where it has not been cleared for potreros. Being an isolated wind-swept and fire-swept peak, the vegetation of the summit has a stunted, subalpine appearance. The Hacienda Granadilla on the plateau between the Río de las Cañas and the Río de San José at about 500–600 m. furnished much interesting material, especially as we were fortunate in finding two huge pochotes (*? Bombax elliptica* HBK.) which had blown over so recently that the crustose lichens were still fresh and the clusters of orchids and bromeliads were still in bloom. These furnished about the only collections to show the flora of the tree tops in Guanacaste.

The region between the Cerro de San José and Puntarenas has not been visited by botanists to my knowledge, although it is quite accessible on account of the Abangares and other gold mines which have been exploited more or less continually since colonial times. Since the region is of a different geological formation it would probably well repay investigation, as would also the calcareous Santa Catalina range between the R. Tempisque and R. Bebedero at the head of the Gulf of Nicoya.

The peninsula of Osa between Golfo Dulce and the Pacific consists of low mountain ranges, probably of sedimentary rock; at least such were the outcrops observed along the shore and in the gorge of the Quebrada de la Laja, a tributary of the Río Nuevo. G. Cufodontis, of the Austrian expedition of 1930, visited the region in the immediate vicinity of Puerto Jiménez (Santo Domingo, on many old maps and charts, on the small bay just above Puntarenitas). Two weeks later I visited the same region, also the Hacienda Sándalo between the Tigre and Terrones Rivers (marked Necki on the charts). The coastal plain about a mile wide slopes gradually from the beach to about 30 m. and then more steeply up the spurs of the range which follows the west coast of the Golfo Dulce. The forest is still virgin in most

places and consists of a rather open palm and hardwood forest characteristic of well-drained areas. The coast between the tide levels has a characteristic mangrove swamp. Mr. Dunlap, of the United Fruit Company, visited this region a few years ago but I have not learned of any specimens taken. Mr. H. J. Marks, the proprietor of the Hacienda Sándalo, has sent a few specimens of wood and herbarium specimens of forest trees to Prof. S. J. Record of Yale University.

Practically nothing is known of the flora of the islands off the west coast. The only one which has been visited by botanists, Isla Coco, has so far yielded very few foliose or fruticose species. Dr. H. K. Svenson, botanist of the Astor expedition, one of the latest to visit the island, describes it as follows: "The lichen flora must be very inconspicuous; I did not go out of my way to look for lichens but if any large forms had been in my path, I believe I would have picked them up. Cocos Island is entirely covered by a rank vegetation which the sunlight rarely penetrates." So far the only species reported are one *Leptogium* and two *Parmelias*, all wide-ranging species of the tropical lowlands.

Botanical Exploration

Of early botanical exploration in Costa Rica the little that is known apparently indicates that few or no cryptogams were collected. Martín Sessé and José Mariano Mociño probably visited the Pacific Coast between 1795 and 1804. The "Sulfur," with George Barclay as botanist, entered the Golfo de Nicoya about 1840 and about the same time Emmanuel Friedrichstahl visited a portion of Nicaragua and Costa Rica though his collections were not carefully labeled as to locality, all the sheets being marked Guatemala.

Between 1845 and 1851, the "Herald" probably visited Pedregal, the harbor for Davíd, Panamá, as Seemann the botanist brought back from Boquete and the neighboring volcano of Chiriquí, lichens which were studied by Churchill Babington and published by Seemann (1852–1857). These specimens are in Babington's herbarium at Cambridge University and in the Royal Botanic Gardens at Kew.

The first to make extensive collections was Anders Sandøe

Ørsted (1816–1872), to whom we owe the first elaborate accounts of the natural history of Costa Rica. For details of his travels and lists of plants collected at various places, see Ørsted (1848, 1851, 1863), Hemsley (1878–1888), and Durand & Pittier (1891). He entered the country at Puntarenas, passing over the old carretera which ascended the Cerros de Aguacate thence via Alajuela to San José, where he made his headquarters for some time. From this point he visited C. Escazú and Pacaca, penetrating to the mountains of Jarís to the south. He also visited Candelaria, probably in the vicinity of Tablazo, judging from the list of plants he collected in that locality. In May, 1847, he explored Poás and Barba. Earlier in that year he had spent some time studying the volcano Irazú, making his headquarters in Cartago. From this point he visited Aguacaliente and Ujarrás (probably the valley below the modern Paraíso), then made the very difficult trip to Moin over the old trail now largely abandoned, passing Cervantes, Río Birrís, and Quebrada Honda to Naranjo (the modern Juan Viñas), thence to Turrialba, keeping along the shelf of land above the lake near Tunnel Camp, Bonilla, etc. to the customs house on the R. Reventazón (probably below the present La Junta) where he crossed the Reventazón in canoes and proceeded along the coastal plain to Moin.

Another long trip was his visit to Guanacaste and adjacent Nicaragua, the details of which were published in 1848 from a letter he had sent home. He does not state in what year this trip was undertaken, but since the mention that Puntarenas was then a free port (this having been decreed in 1847) and since he was visiting volcanoes in February and May, 1847, it is probable that he made the journey in 1848. He was persuaded by government officials to explore a possible route for an inter-oceanic canal between the Lago de Nicaragua and the Pacific. The party, headed by Francisco Gutierrez, left San José on February 17 for Puntarenas, where they took a huge canoe up the Gulf of Nicoya and thence up the Río Tempisque. On March 8 they set out with a pack train from Santa Rosa for the Hacienda Sapoa just above the junction with R. Bolaños. Thence they visited H. Las Animas above the junction with the R. Guachepelín, ascending that valley and descending the R. Tortuga,

the next river east of the R. Sapoa. Ørsted may have visited some of the islands in the Lago de Nicaragua before he ascended the Río Sapoa to the junction of the Sansapote and overland to the H. Sapoa. Another trip was taken to the Golfo de Bolaños, thence over the height of land and down the Sansapote to its junction with the Sapoa.

Ørsted also visited San Juan del Norte (Greytown), crossing the pass between Barba and Irazú at La Palma and descending along the Río Sucio and the R. Sarapiquí. It is not known whether he sailed from San Juan del Norte or from Puntarenas, which was frequently visited by Danish vessels at that period, thirteen having called there in 1853.

Warscewicz, coming from Guatemala via El Salvador and Nicaragua, is said to have met Ørsted in Nicaragua before reaching Costa Rica via the R. Sarapiquí. He evidently visited Irazú and Turrialba, but I have seen no lichens from his collections.

Ørsted's huge collections are to be found in Kjøbenhavn where their study has only recently been completed. They were distributed about 1931. The fungi were sent to Elias Magnus Fries at Upsala and were published promptly by him in 1851, portions of the collections being found in both Kjøbenhavn and Upsala. The lichens were sent to Th. M. Fries but only a single number, *Stereocaulon obesum* Th. M. Fr., was published. Some have since been named and inserted in the herbarium at Upsala. Through the kindness of Professor N. Svedelius, the remaining unnamed specimens were turned over to me for study and are reported in this work. At present none of Ørsted's lichens are at Kjøbenhavn but it is probable that a complete set of duplicates will eventually be deposited in that herbarium.

Ørsted was soon followed in Costa Rica by a number of naturalists who have left us long accounts of geology and natural history. Many resided in Costa Rica for several years, but few appear to have collected lichens.

During his visit, Polakowsky collected a few specimens studied by Nylander (1876) and apparently distributed quite widely, as I have seen a complete set in Berlin and scattered numbers in other herbaria.

A new era for Costa Rican natural history began with the establishment in 1887 of the Instituto Fisico-geografico Nacional de Costa Rica, with Henri Pittier as its first director. Due to his enthusiastic explorations and those of his fellow botanist, Tonduz, a large herbarium was assembled in San José and materials were sent to many foreign collaborators for study, resulting in the publication of Durand and Pittier (1891–1901). The lichens which were studied by Müller Argau were largely from two extensive expeditions to the southwestern portion of the country and scattered numbers collected by others in various parts of the country. Pittier (1891) described these expeditions in detail. As these collections will be frequently mentioned I have thought it wise to include an abstract of their itinerary.

On January 15, the first expedition reached San Marcos de Dota, the capital of the canton of Tarrazú, the next night Santa María de Dota, and the C. del Angelo, small valley of El Copey 1790 m., and El Roble 2670 m., on the following day. On January 18 they passed Alto de la Baraja 2933 m., Rancho de las Vueltas, C. de las Vueltas 3019 m., and Ojo de Agua 2760 m.; on January 19 the Paramo de las Vueltas and the C. de Buena Vista 3299 m., and La Muerte at 3130 m.; on the 20th, southward along the slopes of Buena Vista to Lagunilla 1857 m., the Alto del Palmital at 1211 m., reaching El Géneral on the evening of January 21 where they stayed until January 28, 1891, and whence Biolley returned to San José. Pittier and Tonduz continued to Buenos Aires, Térraba, and Boruca, whence Pittier returned by a somewhat different route to San Marcos and San José, arriving on February 23, while Tonduz stayed in the field until March 8, 1891.

The following season Tonduz left San José on October 18, 1892, more or less retracing the journey of the previous season to Buenos Aires, thence down the R. Grande de Térraba or Diquís, stopping some time at Boruca, finally down the river to the coast and back by sea to Puntarenas, stopping at several points, arriving on April 17, 1893. The material from these two trips furnished the bulk of material upon which Müller Argau (1891, 1893) based his account of the lichens.

These collections are to be found in the Herbier Müller Argau,

which was purchased by the Herbier Boissier and is now at the Université de Genève. I have been told that a duplicate set exists in Brussels, but apparently none was returned to the Museo Nacional in San José.

C. Wercklé, an engineer who resided in Costa Rica for many years, collected much in the vicinity of Cartago. His specimens were determined by Lindau and may be seen in Berlin and in Wercklé's herbarium at the Museo Nacional de Costa Rica. Wercklé was not at all discriminating in selecting samples for Lindau, so that most of the material in San José needed revision.

The Calverts (1917) spent a year, 1909–1910, in Costa Rica and have given us a good account of its natural history. They brought back some plants which are in the herbarium of the University of Pennsylvania, but I have seen only one lichen from them (at the Farlow Herbarium). Maxon and assistants have visited Costa Rica for ferns and brought back some lichens which were sent to G. K. Merrill for study and are now to be found in the Farlow Herbarium (which acquired the Merrill herbarium) and in the National Herbarium of the Smithsonian Institution at Washington.

Paul C. Standley, in 1924 and again in 1925–6, in company with Juvenal Valerio Rodriguez and Ruben Torres Rojas, made very extensive collections of lichens although his primary interest was angiosperms. These lichens were likewise sent to G. K. Merrill who was studying them at the time of his death. As curator of the Farlow Herbarium I continued this study in preparation for my trip to Costa Rica and am here reporting these collections.

In 1925, during a two-weeks' visit, I secured a large amount of material from Cerro Carpintera and again in 1929–30 I secured several thousand specimens of cryptogams which are still being studied. The most complete set of these plants is in my personal herbarium and the next set, lacking only a few numbers of the commoner species, is at the Farlow Herbarium.

Karl Danielson, an assistant to H. E. Stork, in 1928 collected a few numbers of lichens which are now at the University of Michigan. These are also reported here for the first time, duplicates being found in the Farlow Herbarium and in my own herbarium.

In 1929 and 1930 the Costa Rican government botanist, Alberto M. Brenes, collected many lichens in the vicinity of San Ramón and a few from other parts of the country. These were also turned over to me for study and are to be found in the Museo Nacional, with duplicates in my own herbarium and in the Farlow Herbarium.

The Oesterreichische Costa Rica Expedition, under the leadership of Otto Porsch (1932) with G. Cufodontis as assistant botanist, spent the late verano and early invierno in Costa Rica, visiting much the same territory as I did. If lichens were collected they were probably studied by Zahlbruckner but I have seen no publication on this group, although many of the other groups are already published.

Floristics

It is too early to draw any very definite conclusions regarding the distribution of plants and the affinities of the flora, but certain typical distributions are evident.

Families and genera which are abundant in the northern United States and are often widespread in the North Temperate Zone are usually found at the higher altitudes and reach their southern limits in the mountains of Colombia, except for occasional isolated species farther south. Such an example is the Peltigeraceae, which are widespread and abundant in the northern part of the United States. Only two genera, *Nephroma* and *Peltigera*, reach the American tropics. *Nephroma* is represented by a single species seen in only one restricted locality in Costa Rica. *Peltigera* is represented by about eight species, mostly strictly tropical with only one undescribed species reaching Magellanes, Chile.

Another typical example is a northern family, which is characteristic of the Atlantic Coastal Plain, the Collemaceae. Here genera are mostly common to the eastern and western hemispheres but subgenera and species are much more restricted in distribution. These are mostly found on the coastal plains of Costa Rica, being characteristic on the plains of Guanacaste and occasional on the Atlantic Coastal Plain but not yet reported from Panamá. The species belonging in these genera from the

higher elevations have relations with those of the mountains of Colombia and not with those north of the Isthmus of Nicaragua.

A third type of distribution is found in those families which are predominantly southern, as in the Stictaceae. Here we find them highly developed and characteristic in the highlands, relatively rare in the lowlands. The genera and subgenera are world-wide in distribution and the species also have wide ranges, becoming less frequent in numbers and in species in the North Temperate Zone. In this group, however, species of the mountains of Costa Rica may extend into North America along the Atlantic Coastal Plain, even as far as the Avalon peninsula of Newfoundland, while the species of the Coastal Plain in Costa Rica are southern in their ranges.

Finally some cosmopolitan families in which it is difficult to determine a center of distribution have genera or subgenera which are strictly limited to tropical America.

Further consideration of problems of distribution may be deferred until the systematic enumeration is complete. In general the affinities of the flora seem to be with eastern Brasil but since we have very few collections available from the intermediate mountain ranges of eastern Colombia and southern Venezuela and British Guiana, it is likely that stations of many of these species will eventually be discovered in these regions. Relatively few species are common to Mexico and Costa Rica or to Perú and Costa Rica, and practically no Costa Rican species extend as far south as Chile.

In the following systematic enumeration I have included in the keys to species all those which have been described from tropical America, whether they have been found in Costa Rica or not. I have not included species which were not originally described from this area, although they may have been reported from it, since often these reports have been based on misdetermined specimens. While it is evident that political boundaries do not always coincide with natural floristic divisions, for the purposes of this study tropical America may be defined as Mexico and the West Indies southward to Perú, Bolivia, Paraguay, Brasil, and Uruguay. These are quite natural in the present state of our knowledge, as the lichen flora of northern

Mexico which might be expected to show affinities with that of the southwestern United States is practically unknown. Similarly, that of northern Argentina, which might be expected to be similar to that of adjacent Paraguay, has been little explored for cryptogams. Northern Chile, largely desert, has also been little studied, while the flora south of the desert has relatively few species which have been reported from tropical America as here defined. The greatest difficulty occurs in the exclusion of southern Florida where occur many species which are tropical rather than northern in their affinities. Fortunately for our purposes comparatively few species have been described from this region.

KEY TO TROPICAL AMERICAN FAMILIES OF FOLIOSE AND FRUTICOSE LICHENS

Apothecia more or less exposed, paraphyses growing above the asci to form a tissue, asci early evanescent, leaving an enclosed dusty spore mass (mazedium) ..... CONIOCARPINEAE

Thallus foliose or fruticose, corticate, apothecia sessile ..... SPHAEROPHORACEAE

Apothecia linear, elongate-elliptic or angular, not forming a mazedium .....

..... GRAPHIDINEAE

Thallus fruticose, erect or dependent, with a basal layer attached to the substrate, corticate [not yet reported between Lower California and Perú]; maritime ..... ROCCELLACEAE

Apothecia circular, not forming a mazedium ..... CYCLOCARPINEAE

Thallus strictly crustose, but fruiting structures appearing fruticose owing to the proliferation of the apothecia from the margins, easily mistaken for a hepatic on cursory examination; Guiana ..... *Polystroma*

Thallus loosely byssine from filaments of *Cladophora* or *Trentepohlia*, apothecia with light-colored parathecium ..... COENOGONIACEAE

Thallus filiform or dwarf fruticose, or squamose, very rarely small foliose [deferred for further treatment with the crustose species].

Algal symbiont *Scytonema* or *Stigonema*, apothecia more or less sunk in the thallus, small and easily overlooked ..... EPHEBACEAE

Algal symbiont Chroococcaceae ..... PYRENOPSIDACEAE

Algal symbiont *Rivularia* ..... LICHINACEAE

Thallus gelatinous, swelling greatly when moistened, dwarf fruticose, squamose or large foliose, algal symbiont usually *Nostoc* ..... COLLEMACEAE

Thallus definitely foliose or fruticose, or if small, the form of the thallus not due to the algal symbiont.

Hypothallus and rhizoids highly developed, thallus squamose to small foliose, upper surface corticate, algal symbiont usually *Nostoc* .. PANNARIACEAE

Hypothallus evanescent.

Thallus large foliose, algal symbionts Nostocaceae or Palmellaceae, spores fusiform to acicular, 2 or more celled.

Both surfaces corticate, lower surface interrupted by more or less highly developed breathing pores, apothecia with well-developed parathecia or amphithecia, stipitate or sessile.....STICTACEAE

Only upper cortex developed, the lower surface tomentose with more or less highly developed network of veins and tufted rhizoids, apothecia attached to the thallus over their whole under surface without true parathecium or amphithecium although surrounded by the torn remains of the tissue covering the young apothecium.....PELTIGERACEAE

Thallus squamose or small foliose, only upper surface corticate, algal symbiont *Pleurococcus*, apothecia with well-developed light-colored parathecium, spores various.

No secondary thallus or podetia present, apothecia sessile.....PHYLLOPSORACEAE

Secondary thallus or podetia present, varying from simple stalk of apothecium without algae to highly developed branched or infundibuliform structures, primary thallus squamose or small foliose, often evanescent or degenerate, perhaps crustose in some species.....CLADONIACEAE

Thallus large foliose or fruticose, algal symbiont *Pleurococcus*, spores one- or two-celled or muriform, never elongate-fusiform to acicular.

Apothecia with black parathecium, spores one-celled [in *Gyrophora haplocarpa* from Perú, the only species of the family so far reported].....GYROPHORACEAE

Apothecia with bright-colored amphithecia.

Spores not placodiomorphous.

Thallus foliose, spores one-celled.....PARMELIACEAE

Thallus fruticose.....USNEACEAE

Spores placodiomorphous.

Spores hyaline, thallus usually bright yellow, at least the epithecium orange.....THELOSCHISTACEAE

Spores brown, thallus usually more or less glaucous, epithecium brown or pruinose.....PHYSCIACEAE

SPHAEROPHORACEAE

Thallus foliose or fruticose, corticate on both surfaces or the lower surface incompletely corticate, with *Protococcus*. Apothecia sessile on the margin, or on the lower side of the thallus, open at first or enclosed by an amphithecium.

Only two genera have been reported from tropical America, both fruticose with solid axis and terminal apothecium. *Acroscyphus sphaerophoroides* Lév. without an amphithecium has been reported from Mexico and Perú but has not been found in Costa Rica. *Sphaerophorus* is typically Subarctic-Antarctic in distribution, coming southward on the higher mountains with

a single species on the higher mountains of tropical America. The whole family is greatly in need of revision.

SPHAEROPHORUS MELANOCARPUS (Sw.) DC. apud Lam. & DC., Fl. Franç. ed. 2, 6: 178. 1805, excl. syn.

Lichen melanocarpus Swartz, Nova Gen. Sp. Pl. Prodr. 147. 1788. Type: Jamaica, *Swartz*.

Thallus ashy glaucescent or pale above, pale below, partly terete, partly compressed, smooth, not rugose, highly branched, 1–6 cm. high, primary branches 1–4 mm. thick, KOH yellow above, KOH – below, $CaOCL_2$ – ; cortex 20 μ thick, subpellucid, of thick-walled conglutinate irregularly woven hyphae; medulla I –, composed of hyphae 4–8 μ thick; mazedium oblique in the ends of the thicker terete branches, becoming disciform, exciple lacerate, ascospores 7–11 μ in diameter. Spermatogonia immersed in the tips or in the lower surface of the branches, opening by a blackened verruciform mouth; spermatia oblong, 1 x 3 μ [description from Nylander and Vainio].

The Costa Rican material does not altogether agree with the above description, but there is so much variation in macroscopic appearance that only a careful monograph can settle the number of species and their relationships.

Cartago: F. V. Turrialba, 2000–2400 m., *Standley 35313*.

San José: L. la Chonta, n. e. Sta. María de Dota, 2000–2100 m., *Standley 42290;* C. de las Vueltas, 2700–3000 m., *Standley & J. Valerio 43832*.

Heredia: C. Zurquí, 2000–2400 m., *Standley & J. Valerio 50436*.

ROCCELLACEAE

This strictly maritime family has not yet been reported between Lower California and Perú, probably because no suitable maritime habitats have been visited by botanists, for the group is abundant in such strictly tropical habitats as the Galápagos Islands. I did not have an opportunity to visit such myself, but had I been able to visit the cliffs along the various headlands between Puntarenas and Golfo Dulce, it is possible that I might have secured some representatives of this family.

COENOGONIACEAE

Thallus spongy byssoid, either adnate or forming dimidiate shelving masses, homoeomerous with *Trentepohlia* or *Cladophora*,

whose filaments are partially surrounded by hyphae. Apothecia with pseudoparenchymatous parathecium; asci 8-spored; spores hyaline, one- or two-celled; spermatia exobasidial.

Thallus with *Trentepohlia* ..... *Coenogonium*
Thallus with *Cladophora*, apothecia unknown ..... *Racodium*

COENOGONIUM Ehrenb.

COENOGONIUM Ehrenberg apud Nees ab Esenbeck, Horae Physicae Berol. 120. 1820.

Thallus loosely spongy byssoid, either adnate or forming dimidiate shelving masses (suggesting a thin species of *Polystictus*), homoeomerous with *Trentepohlia* which is partially surrounded by a network of hyphae. Apothecia scattered on the upper surface, scutiform, usually with a short stipe, with a parathecium of thin-walled pseudoparenchyma without a medulla; paraphyses unbranched, often with swollen tips; asci 8-spored; spores hyaline, fusiform, ellipsoidal to elongate, one- or two-celled; spermagonia spherical, spermatia exobasidial, fusiform, straight; spermatiophores mixed with anaphyses.

Clements segregated the species of *Coenogonium* with unicellular spores as *Holocoenis*. However, his proposal has not been followed by other authors, although Vainio and Zahlbruckner have proposed sections on this basis, Vainio using *Coenobiatora* and *Coenobiatorina*, while Zahlbruckner incorrectly adopted *Holocoenis* and *Coenobiatorina*.

This family is very aberrant among lichens in several respects and perhaps would be better dropped and the species distributed among the fungi and algae. It seems more logical to regard the group as a case of parasitism of fungi on algae, as neither fungus nor alga seems to influence the development of the other, and there has been no further evolution resulting from the acquisition of a photosynthetic unit by the fungus. Taking this view, one would recognize about four or perhaps five species of fungi from tropical America, one species with unicellular spores about 6–10 x 2.5–4 μ belonging in *Patinella;* and three or four species with 2-celled spores belonging in *Orbilia* or a segregate from that genus. Among the algae one would recognize a dozen or more species of *Trentepohlia* which are often subject to the attacks

of *Patinella* and *Orbilia*. In the following key to the tropical American species of *Coenogonium*, as it is commonly understood by lichenologists, it will be noted that out of nineteen names proposed, four were based on specimens in which no apothecia were found. Four other species originally described in *Coenogonium* have already been transferred to *Trentepohlia*. I have carefully investigated the ascocarps of the fertile Costa Rican specimens and can find few characters which would separate species from each other, except spore size and septation. For lack of time to investigate this question thoroughly, I have here followed the traditional arrangement, as to do otherwise would involve serious nomenclatorial difficulties. To adopt the view that this group should be excluded from the lichens would invalidate *Coenogonium*, as based on a combination of characters of parasite and host and involve redescription of its species in *Patinella*, *Orbilia*, and *Trentepohlia*.

KEY TO THE TROPICAL AMERICAN SPECIES OF COENOGONIUM

Apothecial disc livid, cell walls brownish; spores unicellular; algae 11–16 μ in diameter; French Guiana ..... *C. Leprieurii*

Apothecia carneous, yellowish or waxy white, never livid.

Thallus dimidiate, not adnate to the substrate.

Thallus thick, rigid, with white subsilky villum, zonate, sterile; Brasil .. .. *C. Echinus*

Thallus thinner.

Filaments 7–9 μ in diam., cells not very distinct, greenish, subdiffuse, stratose ..... *C. subvirescens*

Filaments 12 μ in diam., rigid; Brasil ..... *C. Linkii*

Filaments thicker, 16–20 μ in diam., thallus less rigid.

Cells 4–5 times as long as broad; Mexico ..... *C. confervoides*

Cells about twice as long as broad; spores 7–8.5 x 2–3 μ; Brasil .... *C. acrocephalum*

Filaments and paraphyses thick, imperfectly known ..... *C. andinum*

Thallus adnate to effused.

Filaments moniliform, 18 μ in diam.; thallus glaucous green to tawny brown, margins paler; spores 2-celled, 3–4 times as long as broad; Cuba ..... *C. moniliforme*

Filaments partially moniliform, partly cylindrical, cells 20–25 μ in diam.; Costa Rica ..... *C. heterotrichum*

Filaments uniformly cylindrical.

Filaments 23–36 μ in diam.; striate apothecia 0.6–0.7 mm. in diam.; spores 11–15 x 25–35 μ, 2-celled ..... *C. disjunctum*

Filaments 20–28 μ in diam.; spores unicellular, 6–10 x 3 μ; Bourbonia or Louisiana ..... *C. interpositum*

Filaments 17–20 μ in diam.; cells 30–50 μ long; spores 8 x 2.5 μ, 2-celled; Costa Rica ..... *C. interponendum*
Filaments 15–17 μ in diam.; thallus ashy green, pulvinate, up to 2 cm. broad; Perú ..... *C. pulvinatum*
Filaments 12–16 μ in diam.; thallus yellowish; spores 8–10 x 3 μ; Colombia ..... *C. interplexum*
Filaments 12–14 μ in diam.; thallus yellowish to green; St. Vincent ..... *C. Leprieurii* v. *panniforme*
Filaments 8–11 μ in diam., conglutinate in fascicles 70–90 μ in diam.; spores 7–10 x 3.5–4.5 μ; Bolivia ..... *C. complexum*
Filaments 4–8 μ in diam.
Filaments non-articulate; spermatia 7–9 x 15 μ; paraphyses clavate; Brasil ..... *C. dialeptizum*
Filaments articulate; spermatia not reported.
Cells 2.5–4 times as long as broad, paraphyses obovoid; Brasil ..... *C. pannosum*
Cells 1.5–3 times as long as broad, sterile; Brasil ..... *C. depressum*
Filament size not given, rugulose, articulate-branched, coalesced into fascicles; Venezuela ..... *C. Tuckermani*

COENOGONIUM LEPRIEURII Nyl., Ann. Sci. Nat. Bot. IV, **16**: 89. 1862.

C. Linkii var. *Leprieurii* Mont., Ann. Sci. Nat. Bot. III, **16**: 47. 1851.

Holocoenis Leprieurii Clements, Genera of fungi, 174. 1909.

Type: Guayane Française, *Leprieur*, sine no.

Algae loosely woven, yellowish, 11–16 μ in diameter. Apothecia plane or convex, excluding the white margin, disc livid from the first; asci about 20 μ in diameter; spores oblong to fusiform or ellipsoidal, unicellular, 6–10 x 2.5–4 μ; paraphyses slender, apices clavate.

I have not seen this species from Costa Rica but *Dodge & Nevermann 7399*, from jungle at Castilla farm, 20 m., Limón Province, seems to be a variety of this species. The fungus is not well developed but has a livid disc, the margin is still thick and white, and the alga is of much smaller diameter. The exciple is composed of isodiametric cells with colored walls. It appears to be the same fungus attacking a much smaller alga, and should be referred to *Patinella*.

COENOGONIUM SUBVIRESCENS Nyl., Flora **57**: 72. 1874.

C. Leprieurii var. *subvirescens* Nyl., Ann. Sci. Nat. Bot. IV, **16**: 89. 1862.

Type: Brasil, Amazonas, Rio Negro, *Spruce 28*, is type of the

species, while *C. Leprieurii* var. *subvirescens* was described from French Guiana without collector.

Algae somewhat interwoven but more or less parallel, 7–9 μ, forming a loose dimidiate to subcircular thallus depending upon position and shape of substrate, not or obscurely zonate, pale to dark olive buff, cells about 21–24 μ long, septa not very evident, other cell walls thick, chloroplasts appearing somewhat in the shape of a dumb-bell with the pigment more or less massed at the ends of the cells.

Apothecia about 800 μ in diameter, tapering below to a short stipe about 250 μ in diameter, composed of thick-walled pseudoparenchyma with the outer 40 μ composed of a palisade of elongate cells, perpendicular to the surface; parathecium about 80 μ thick, homogeneous with the rest of the apothecium, the hypothecium very thin, filamentous, of cells with thinner walls. The hymenium is about 80 μ tall and is composed of filiform paraphyses with clavate tips and slender clavate asci. The ascospores are not mature in my specimens but seem to be slender, 2-celled, probably about the size of most of the other spores in the genus except *C. disjunctum*.

Limón: Cadiz, *Dodge 7452;* Castilla farm, 20 m., *Dodge & Nevermann 7451;* Monte Verde, *K. Danielson 73;* Siquirres, 70–200 m., *Dodge, Catt & Thomas 5574;* La Colombiana, 70–80 m., *Standley 36885.*

Cartago: R. Pejivalle, 650–800 m., *Dodge & Thomas 4408;* El Muñeco, on R. Navarro, 1400–1500 m., *Standley & J. Valerio 51055;* C. Carpintera, 1500–1850 m., *Standley 35596.*

Puntarenas: Osa, Golfo Dulce, *Dodge 7450.*

Guanacaste: Q. Serena near Tilarán, 700 m., *Standley & J. Valerio 46242;* Santamaría, 720–850 m., *Dodge 7015.*

COENOGONIUM LINKII Ehrenberg ap. Nees ab Esenbeck, Horae Phys. Berol. 120. 1820.

C. controversum Pers. ap. Gaudich. in Freycinet, Voy. Uranie, Bot. 214. 1826 (nom. nud.).

Type: Brasil, Sta. Catharina, *Chamisso.* The type of *C. controversum* came from the walls of the aqueduct of Corcovado, Rio de Janeiro, *Gaudichaud.*

Algae somewhat interwoven but more or less parallel, about 12 μ in diameter, forming a somewhat rigid dimidiate to suborbicular thallus, not or obscurely zonate, pale to dark olive-buff, cells 40–60 μ long, septa very difficult to observe, chloro-

plasts small and scattered, outer cell walls thick, partially covered with anastomosing fungal hyphae.

Apothecia warm buff, about 850 μ in diameter, tapering sharply to a short stipe about 240 μ in diameter and up to 300 μ long, pseudoparenchymatous, without palisade layer at the surface; parathecium about 100 μ thick, homogeneous with the rest of the apothecium, the hypothecium very thin and soon evanescent; the hymenium about 80 μ tall, composed of filiform paraphyses with swollen tips and cylindrical asci. The ascospores are not fully mature in my specimens but probably the common size for the genus.

Cartago: C. Carpintera, 1800 m., *K. Danielson 9.*
Alajuela: La Palma de S. Ramón, 1100 m., *Brenes 175.*
Guanacaste: El Silencio, near Tilarán, 750 m., *Standley & J. Valerio 44619.*

COENOGONIUM CONFERVOIDES Nyl., Flora **41**: 380. 1858.
?*C. andinum* Karsten ap. Nyl., Bot. Zeit. **20**: 178. 1862.

Type: Mexico, Orizaba, *Fr. Müller* (Schimper Herb.). Type of *C. andinum* from Colombia, 2000 m., *Lindig 2560*, also Perú, *Weddell*, and southern Brasil, *Guillemin*. Since I have been unable to see the types, the interpretation of this species is difficult. In Ann. Sci. Nat. Bot. IV, **11**: 242. 1859, Nylander amplified his very inadequate description, basing his emendations on a specimen from Tahiti, *Lépine 14*. When he monographed the genus in Ann. Sci. Nat. Bot. IV, **16**: 91. 1862, he cited *Lepine 14* from Tahiti first, adding Guadeloupe Island, *Duchassaing*, Brasil, *Gaudichaud*, and *Weddell*, and Chile, *Gay*, not citing the original Mexican type. Since none of these descriptions was based on specimens having apothecia its application is further confused. Since the descriptions only state how it differs from *C. Linkii*, I have assumed that the thallus is more or less dimidiate with a larger alga than in that species. Nylander also notes that a specimen from Colombia, Bogota, 2700 m., *Lindig 887*, has algal filaments 12–18 μ in diam. Unfortunately I have been unable to see any of the above-mentioned specimens. The following description is based on Costa Rican specimens.

Algae somewhat interwoven but more or less parallel, 16–20 (–28) μ in diameter, forming a soft dimidiate or suborbicular thallus, not zonate, pale to deep olive buff, cells about 60–100 μ

long, septa plainly visible, chloroplasts rather indistinct in these specimens, often alternate cells more or less collapsing, giving a somewhat moniliform appearance to old filaments, outer walls thick, not densely covered with hyphae. The collapsed cells sometimes have the coloring matter collected at the ends, giving the dumb-bell appearance to the chloroplast as in *C. subvirescens.*

Apothecia warm buff, 560 μ in diameter, patelliform, with stipe about 160 μ in diameter and 300 μ long, pseudoparenchymatous, of very thick-walled cells which tend to arrange their long diameters perpendicular to the surface of the apothecium, but not forming a definite palisade as in *C. subvirescens;* the parathecium 40–50 μ thick, homogeneous with the rest of the apothecium, the hypothecium 20–30 μ thick of interwoven hyphae, the hymenium about 60 μ tall, composed of filiform paraphyses with spherical tips and slender cylindrical asci about 5 μ in diameter; ascospores distichous, 4-celled, 10–12 x 3 μ. [The ascospores are immature and so closely packed in the ascus that I am not certain of size and septation, as I have been unable to free spores from the ascus.]

Limón: Waldeck, *Dodge 7453.*

Cartago?: Morpho Valley, 1420 m., *K. Danielson 51* [This locality is uncertain and Danielson's barometric readings are apt to be a little high, but it probably in the vicinity of the upper Reventazón below Cartago, where Lepidoptera of the genus *Morpho* are not uncommon.]

COENOGONIUM HETEROTRICHUM Müll. Arg., Bull. Soc. R. Bot. Belg. **32**: 162. 1893.

Type: Costa Rica, San Marcos de Dota, *Tonduz 6115.*

Thallus pulvinate, cespitose-effused, deep olive buff to dark olive, algae somewhat interwoven, dimorphic, larger filaments 20–25 μ in diameter, cells about 40 μ long, chloroplasts disciform, scattered; smaller filaments submoniliform, the cells of smaller diameter being about 7–8 μ and 12 μ long, those of larger diameter being about 10–12 μ and of about the same length.

Fungus unknown. In *Brenes 29a* the fungal hyphae are brown instead of hyaline.

San José: S. Marcos de Dota, 1200 m., *Tonduz 6115,* type.

Alajuela: La Palma de S. Ramón, 1250 m., *Brenes 29a.*

COENOGONIUM INTERPONENDUM Nyl. ap. Polakowsky, Jour. Bot. Brit. & For. **15**: 225. 1877.

Type: Costa Rica, Cartago, Angostura, *H. Polakowsky 496*.

Algae loosely interwoven, forming an adnate irregular thallus, deep olive buff to citrine drab, cells about 17–20 μ in diameter, 30–50 μ long, pigment quite uniformly dispersed throughout the cell, yellowish green. All my Costa Rican material is sterile. Nylander states that the spores are fusiform, 2-celled, 8 x 2.5 μ.

Limón: Carmen, *Dodge 7449*.
Cartago: Angostura, *H. Polakowsky 496*, TYPE.
Alajuela: La Palma de S. Ramón, 1250 m., *Brenes, 53a, 116, 399*.
Guanacaste: H. Q. Azul on lower slopes of V. Tenorio, 400–600 m., *Dodge & Thomas 8042*.

COENOGONIUM INTERPOSITUM Nyl., Ann. Sci. Nat. Bot. IV, 16: 91. 1862.

Type: Borbonia, Lepervanche, *Mezières;* Louisiana, *Hale*.

Thallus loosely tomentose intertangled, more or less pulvinate, algal cells 20–28 μ in diameter and 60–88 μ long, chloroplasts appearing the shape of a dumb-bell with the pigment massing at the ends of the cells.

[Apothecia pale carneo-luteous; spores oblong, simple, 6–10 x 3 μ; paraphyses slender or medium.]

As all my Costa Rican material is sterile the reference of it to this species is doubtful. It quite possibly belongs with *C. disjunctum* Nyl., based on specimens from Martinique and Cuba, *Wright 170*, which reaches a slightly larger diameter of algal filament and much larger 2-celled spores. Both species are said to resemble *C. confervoides* and perhaps should be placed in the *C. confervoides* group.

Cartago: C. Carpintera, 1560–1700 m., *Dodge & Thomas 7920*.
Alajuela: La Palma de S. Ramón, 1100–1250 m., *Brenes 53, 115*.

COENOGONIUM INTERPLEXUM Nyl., Ann. Sci. Nat. Bot. IV, 16: 92, *pl. 12, f. 20, 21*. 1862.

Type: Colombia, 2200 m., *Lindig 2561*.

Thallus loosely interwoven, intricate, algal cells 12–16 μ in diameter and 30 μ long, chloroplasts more or less disciform, scattered.

[Apothecia fleshy-yellow, plane, 1 mm. in diameter, margin waxy, fleshy to white; spores short-fusiform, 2-celled, 8–10 x 3 μ; paraphyses medium, apex clavate.]

Alajuela: Piedades de S. Ramón, 900 m., *Brenes 408*.
Puntarenas: Boruca, 560 m., *Tonduz 6114*.

COENOGONIUM PANNOSUM Müll. Arg., Flora **64**: 234. 1881.

Type: Brasil, São Paulo, Apiahy, *Puiggari 1026*.

Thallus loosely interwoven, dark olive buff to citrine drab, more or less adnate to substratum, algal cells 6–8 μ in diameter, 28–32 μ long, pigment migrating toward the ends of the cells.

[Apothecia 350–500 μ in diameter, pale or orange white, plane, with paler and thinner margin, finally immarginate and slightly convex, becoming more flesh color; hymenium hyaline, paraphyses very slender with obovoid head about three times as thick as the stalk; asci slender, cylindrical, 8-spored; spores 2-locular, 6–7 x 2 μ, fusiform with acute ends.]

Since my Costa Rican material is sterile its reference here is uncertain and it may be only very young algae of *C. subvirescens* Nyl., with which it agrees in general appearance, but there is no trace of the formation of a dimidiate thallus.

Guanacaste: H. Q. Azul, on slope of V. Tenorio, 500–600 m., *Dodge & Thomas 6654*.

COENOGONIUM DEPRESSUM Müll. Arg., Flora **64**: 525. 1881.

Type: Brasil, São Paulo, Apiahy, *Puiggari 1034*.

Thallus adnate, pale olive buff, filaments short, flexuous, depressed, subintricate; cells 4–8(–13) μ in diameter, 16–20 μ long, somewhat inflated in the middle but scarcely enough to give a moniliform appearance, chloroplasts no longer distinct.

As I have not compared the Costa Rican material with the type I cannot be certain of the identity.

Cartago: alpine region of Irazú, *Ørsted*.

COENOGONIUM IMPLEXUM Nyl., Ann. Sci. Nat. Bot. IV, **16**: 92. 1862.

Type: Australia, Victoria, Jarvin, *Ferd. Müller*.

[Similar to *C. interplexum* but algal cells a little smaller, 10–13 μ in diameter, spores a little larger, 8–11 x 3.5–4.5 μ, paraphyses thicker.]

The following collection has been referred here by Müller Argau, but I have not studied it.

Puntarenas: Boruca, 560 m., *Tonduz 6113*.

EPHEBACEAE

Thallus dwarf fruticose, branched, more or less filiform, without rhizinae, crustose or small squamose, with *Scytonema*

or *Stigonema*. Apothecia small, often with very small punctiform disc, scarcely visible; paraphyses well developed or absent; asci 8-spored; spores hyaline, 1–2-celled.

The individuals of this family are so small that they are easily overlooked both in collecting and in sorting material, and so the few species here reported are probably not representative of this group in Costa Rica.

KEY TO TROPICAL AMERICAN GENERA OF EPHEBACEAE

Thallus crustose to small squamose, homoeomeric; Brasil.....*Pterigyopsis atra*
Thallus dwarf fruticose, dark, thickly branched.
Apothecia sunken in swellings of the thallus, single or gregarious; spores unicellular; paraphyses present; Brasil.....*Ephebeia*
Apothecia sessile on the thallus, either lateral or terminal.
Thallus without pseudoparenchymatous cortex or medulla; paraphyses filiform, simple; asci 8-spored; spores ovoid or spherical, unicellular .....*Thermutis*
Thallus with pseudoparenchymatous cortex and medulla.
Spores unicellular.....*Leptogidium byssoides*
Spores 2-celled.....*Polychidium*

THERMUTIS E. Fr.

THERMUTIS E. Fr., Syst. Orb. Veg. **1**: 392. 1825.

Gonionema Nyl., Mem. Soc. Sci. Nat. Cherbourg **3**: 163. 1855.

Thallus dwarf fruticose, thickly branched and filiform, without rhizinae, *Scytonema* present with the hyphae running in the gelified sheath. Apothecia small, lateral, saucer-shaped to almost spherical; parathecium often highly developed and thick; hypothecium light-colored; paraphyses unbranched, filiform, tips not swollen; asci clavate, thin-walled, 8-spored; spores hyaline, ellipsoidal, elongate, unicellular, thin-walled. Spermagonia lateral or terminal, sessile, more or less spherical; spermatia small, ovoid, or elongate.

THERMUTIS VELUTINA (Ach.) Fw., Linnaea **23**: 170. 1850.

Lichen velutinus Ach., Lichenog. Suec. Prodr. 218. 1798.

Gonionema velutina Nyl., Act. Soc. Linn. Bordeaux **21**: 262. 1856.

This species has been reported by Müller Argau from the following localities in Costa Rica. The specimens were not studied while I was in Genève.

San José: San Marcos de Dota, 1200 m., *Tonduz 5378.*
Guanacaste: Boruca, 560 m., *Tonduz 5371;* Térraba, *Tonduz 5372.*

A small sterile specimen from Guanacaste: near Tilarán, 500–690 m., *Dodge & Thomas 8031*, may belong in *Polychidium* but the specimen is not definitely determinable as to genus.

COLLEMACEAE

Thallus gelified, crustose to foliose or dwarf fruticose, with or without rhizoids, sometimes umbilicate, homoeomerous with *Nostoc*. Apothecia from sunken almost perithecia to sessile apothecia usually with an amphithecium, occasionally with parathecium; paraphyses simple; asci 8-spored; spores hyaline, spherical to acicular, straight or twisted, 1-celled to muriform, usually with a thin wall (except in *Physma* and section *Lemphospora* of *Lempholemma*).

This family is widely distributed in the temperate and tropical zones, the more highly developed members occurring in the latter. It is possible that some of the smaller and less conspicuous genera have been overlooked or have been included in the crustose material in the preliminary sorting. So far only four genera have been found in Costa Rica.

KEY TO TROPICAL AMERICAN GENERA OF COLLEMACEAE

Spores unicellular.
- Thallus crustose, not gelified, parathecium present; spores ellipsoidal, thin-walled.
  - Brasil ..... *Leprocollema americanum*
  - Porto Rico ..... *L. Finkii*
- Thallus squamulose, gelified; spores spherical, thin-walled but surrounded by a thick gelified sheath.
  - Thallus not corticate ..... *Lempholemma*
  - Thallus corticate, wholly pseudoparenchymatous; Juan Fernandez .... *Lemmopsis polyschidioides*
  - Thallus foliose, gelified; spores ellipsoidal to fusiform, thick-walled, surrounded by a gelified sheath ..... *Physma*

Spores phragmospores or dictyospores.
- Cortex not developed; apothecia with parathecia only ..... *Collema*
- Cortex of pseudoparenchyma; apothecia with amphithecia (parathecia often also present) ..... *Leptogium*

LEMPHOLEMMA Körb.

LEMPHOLEMMA Körber, Syst. Lich. Germ. 400. 1855.

Type species: *Lempholemma compactum* Körber.

Thallus from verrucose, squamulose, dwarf fruticose to foliose in our species, gelified when moist, clothed with rhizinae below,

homoeomeric, without cortex, with *Nostoc*. Apothecia superficial or terminal, mostly sunken in the thallus (not in our species); amphithecium either with or without cortex; parathecium colorless, urceolate; hypothecium colorless; paraphyses filiform, simple; asci 8-spored, clavate, often twisted below; spores hyaline, fusiform, ellipsoidal-ovoid or spherical, smooth.

The genus has been divided into eight sections of which only one section *Lemphospora* is tropical. This section is characterized as having apothecia without cortex, spores more or less spherical with a thick gelified sheath. *Lempholemma Dussii* (Vainio) Zahlbr., Cat. Lich. Univ. **3**: 23. 1925 [*Collema Dussii* Vainio, Ann. Acad. Sci. Fenn. A6[7]: 114. 1915] has been described from Guadeloupe in the Antilles.

LEMPHOLEMMA (LEMPHOSPORA) **oblique-peltatum** (Eschw. ap. Martius) Dodge, comb. nov.

Collema oblique-peltatum Eschw. ap. Martius, Icon. Pl. Cryptog. Fasc. 2: 27, *pl. 11, f. 2* [between 1828 and 1833].[1]

Type: Brasil, near Pará, *Martius*.

Thallus foliose, up to 5 cm. in diameter, drying grayish olive to dark olive gray, margin thick, broadly subdichotomously lobed, lobes up to 3 mm. broad and 2–3 mm. long, smooth, surface minutely pitted although appearing practically smooth to the naked eye, without soredia or isidia; thallus 200–400 μ thick, homoeomerous, without cortex, but provided with a thin layer of tangled rhizinae below. Apothecia subsessile, often appearing obliquely attached to the thallus in section, up to 2.5 mm. in diameter, margin of the same color as the thallus, with periclinal folds and wrinkles, disc tawny to mars brown; amphithecium appearing lobulate in section (due to the periclinal wrinkles), homogeneous with the thallus, 200–300 μ thick but extending beyond the parathecium as much as 800 μ; parathecium pseudoparenchymatous, highly developed, about 100 μ thick below, thinning out above to about 50 μ; hypothecium about 40 μ thick, of highly gelified hyphae; thecium about 100 μ

[1] I have been unable to locate the date of the second fascicle of this work. The cover of the copy in the Missouri Botanical Garden gives the pages of text and plates in each fascicle but only the dates 1828–1834. Martius in his 'Flora Brasiliensis' **1**: 233–234. 1833, quotes from the 'Icones' citing page, hence it must have been issued before that date. In this work he placed it in the subgenus *Enchylium*.

tall; paraphyses about 1–2 μ in diameter, clavate above, septate, cells 4–6 μ long, with walls highly gelified so that they appear as slender rows of cells imbedded in a gel; asci clavate-cylindrical, about 80 x 16 μ, containing 8 monostichous spores; ascospores broadly ellipsoidal, 12 x 8 μ while still in the ascus, with a thick gelified wall.

While I have not studied the type specimen of this species, a careful study of the description and the figures shows that it belongs in *Lempholemma* section *Lemphospora* rather than in *Collema*. Judging from determinations observed in various herbaria, this species seems to have been confused with *Leptogium vesiculosum* (*L. bullatum*), *L. tremelloides*, and *L. foveolatum*, although the abundant development of rhizoids below should have separated it. While it is minutely scrobiculate-foveolate (visible under hand-lens), it is not conspicuously so to the naked eye as in *L. foveolatum*. Besides my Costa Rican material I have also seen it from Eustis, Lake Co., Florida, *Nash 2024*, determined by Eckfeldt as *L. faveolatum* (sic) Nyl.

Limón: Waldeck, *Dodge 7404;* near Siquirres, 70–170 m., *Dodge, Catt & Thomas 5588, 8024;* Hamburg, 55 m., *Standley & J. Valerio 48767.*

Guanacaste: H. Santamaría, 720 m., *Dodge & Thomas 6898;* near Tilarán, 340–670 m., *Dodge & Thomas 6644, 8022.*

Puntarenas: near Corozál, 5–50 m., *Dodge 8023;* Puerto Jiménez, *Brenes 839.*

LEMPHOLEMMA (LEMPHOSPORA) **dichotomum** Dodge, sp. nov. Type: Costa Rica, Guanacaste, H. Granadilla, *Dodge & Thomas 6736.*

Thallus foliosus, ad 5 cm. diametro, 400–650 μ crassitudine metiens, griseo-olivaceus, margine crassiuscula, integra, lobatus, dichotomus, lobi 1–1.5 mm. lati, divergentes, superne longitudinaliter rugosus, sine sorediis isidiisque, inferne rhizinis nigris densissimis intertextis obsitus, homoeomerus, nostocaceus, sine strato corticali. Apothecium peltatum, basi constrictum, ad 2.5 mm. diametro metiens, margine concolori, rugosa, disco fulvo castaneove; amphithecium sectione lobatum, homogeneum, 150–300 μ crassitudine inferne, sed ad 500 μ superne et ultra marginem parathecii; parathecium pseudoparenchymaticum, ad 160 μ crassitudine inferne, attenuatum ad 80 μ superne; hypothecium filamentosum, ad 80 μ crassitudine, hyphis gelifactis; thecium ad 120 μ altum; paraphyses filiformes, apicibus

decompositis brunneis, in materia gelata fixi, cellulis cylindricis, 1 μ diametro, 4–6 μ longitudine metientibus; asci 60 x 10 μ, cylindrici; ascosporae octonae, monostichae, late ellipsoideae, 8 x 12 μ (dum in ascis sunt), membrana incrassata, gelifacta.

Thallus foliose, up to 5 cm. in diameter, 400–650 μ thick, grayish olive, margin thick, smooth, dichotomously lobed, lobes 1–1.5 mm. broad, divergent, upper surface longitudinally wrinkled, without isidia or soredia, lower surface covered with a dense black nap of rhizinae, homoeomerous, with *Nostoc*, without cortical layer either above or below. Apothecium constricted at the base, up to 2.5 mm. in diameter, margin concolorous, irregularly wrinkled, disc tawny to chestnut; amphithecium in sections appearing lobed, homogeneous with the thallus, 150–300 μ thick below but extending up to 500 μ above and beyond the margin of the parathecium which is pseudoparenchymatous, about 160 μ thick below, thinning out to 80 μ at the margin; hypothecium filamentous, 80 μ thick, of gelified hyphae; thecium about 120 μ tall, paraphyses filiform, the apices decomposing brown, imbedded in a gel, cells cylindric, about 1 μ in diameter, 4–6 μ long; ascospores 8 per ascus, monostichous, broadly ellipsoidal, about 8 x 12 μ (while still in the asci), with a thick gelified wall. This species has also been seen from Barro Colorado Island, Gatun Lake, Panamá.

Guanacaste: H. Granadilla, 540 m., *Dodge & Thomas 6737*.

PHYSMA Mass.

Physma Massalongo, Neag. Lich. 6. 1854.

Dichodium Nyl., Bull. Soc. Linn. Normandie II, **2**: 43. 1868.

Type: *Physma Boryanum* Massalongo.

Thallus foliose with rhizinae beneath, corticate with several layers of pseudoparenchymatous cells; algae *Nostoc*. Apothecia superficial, lecanorine, with broad disc, thick margin; hypothecium light-colored; paraphyses filiform; asci 8-spored; spores colorless, ellipsoidal or fusiform, unicellular with thick almost warty wall or with a gelified sheath. Spermagonia sunk in the thallus, showing above by the dark swelling, surrounded by pseudoparenchyma; spermatiophores simple or forked, septate, cells short; spermatia short, straight.

Only two species are known from tropical America, *P. chilensis* Hue from Chile and *P. pruinosum* Vainio, from the Antilles. Material reported from America as belonging to *P. byrsinum* (Ach.) Müll. Arg. (*P. byrsea* (Ach.) Tuck.) is probably misdetermined. Apparently the genus is most highly developed and wide-spread in Oceania and adjacent Asia.

PHYSMA PRUINOSUM Vainio, Ann. Acad. Sci. Fenn. A6[7]:112. 1915.

A single sterile specimen collected near Cartago, 1500 m., *C. Wercklé*, May 1900 (in Mus. Nac. 17233, and in Herb. Bot. Gard. Berlin), was referred to *Physma byrsinum* by Lindau. It is possible that this species belongs to *Physma pruinosum* Vainio or it may possibly be a very young thallus of *Leptogium marginellum* (Sw.) S. F. Gray. Its spermagonia are marginal and its spermatia are ellipsoidal and small.

COLLEMA Wigg.

COLLEMA Wiggers, Primit. Fl. Holsat. 89. 1780.

? *Gabura* Adanson, Fam. Pl. **2**: 6. 1763?

Scytenium S. F. Gray, Nat. Arrang. Brit. Pl. **1**: 398. 1821.

Type species: *Collema Lactuca* (Web.) Wiggers [*Lichen crispus* L.].

Thallus foliose or squamulose to almost crustose, gelified when moist, lying on the substrate without rhizinae, homoeomerous, not corticate, hyphal system loose; algae *Nostoc*. Apothecia at first sunken, erumpent, sessile or scutellate and constricted below, with amphithecium; parathecium either present or absent, both parathecium and hypothecium either of interwoven hyphae or pseudoparenchymatous; paraphyses simple, adherent, mostly septate; asci 8-spored; ascospores colorless, cylindric, acicular, fusiform, long-ellipsoidal or ovoidal to almost cubical, ends obtuse or acute, sometimes becoming muriform, thin-walled, without gelified sheath. Spermagonia sunken in the thallus or in thalline warts, with light-colored wall; spermatiophores simple or branched, without sterigmata, septate with short cells; spermatia short, oblong to ellipsoidal, straight.

KEY TO TROPICAL AMERICAN SPECIES OF COLLEMA

Amphithecium corticate; spores acicular ..... COLLEMODIOPSIS

[*C. lherminieri* Hue, from Guadeloupe, is the only species of this typically northern group so far reported. *C. Granadillae* is here reported from Costa Rica.]

Amphithecium not corticate.
Spores oblong to ellipsoidal, more or less muriform; thallus laciniate, more or less granular.....BLENNOTHALLIA
Lobes less than 0.5 mm. broad; apothecia unknown; Chile.....*C. millegranum*
Lobes more than 0.5 mm. broad; apothecia well developed; Mexico.....*C. mexicanum*
Spores clavate, ellipsoidal to fusiform, uniseptate, rarely up to 3-septate, not over 30 μ long; thallus narrowly laciniate or incised.....DICOLLEMA
Spores clavate, 14–21 x 4.5–5.5 μ; Brasil, Paraguay, and Argentina.....*C. corynesporum*
Spores ellipsoidal or fusiform.
Margins of laciniae nodose-granular.....*C. pycnocarpum*
Spores 6–9 x 3 μ; Brasil.....v. *Minarum*
Spores 10–14 x 3–4 μ; Brasil.....v. *Malmei*
Spores 10–15.5 x 4–5.5 μ; Brasil and Colombia.....v. *crassiusculum*
Spores 14–17 x 4–5 μ; New York and New Jersey.....v. *typicum*
Spores 16–25 x 3–7 μ, 3-septate; Virginia southward.....*C. cyrtaspis*
Spores 16–30 x 4–5 μ, 1–3-septate; Chile.....*C. pycnocarpoides*
Margins of laciniae not nodose-granular.
Laciniae 1–3 mm. broad, spores 12–14 x 5–6 μ; Paraguay.....*C. crenatum*
Laciniae under 1 mm. broad, more or less canaliculate below; Cuba.
Laciniae thick.....*C. stellatum*
Laciniae thin, flat; spores 11–17 x 4–5 μ.....*C. solenarium*
Spores fusiform to acicular, more than 3-septate, over 30 μ long; thallus broadly laciniate, often fenestrate.....SYNECHOBLASTUS
Thallus isidiose; spores over 100 μ in length; Brasil.
Parathecium present, spores 125–175 x 3–4 μ.....*C. leptosporum*
Parathecium absent, spores somewhat over 100 μ in length.....*C. Ramboi*
Thallus not isidiose; spores not over 95 μ in length.
Apothecia white-pruinose.
Apothecia small, crowded on short bullate prominences; spores 50–80 x 3–5 μ; thallus pustulate, not fenestrate; southern United States and southward along coastal plains.....*C. leucopepla*
Apothecia sessile or nearly so, larger; thallus more or less fenestrate; tropical highlands.....*C. glaucophthalmum*
Spores 77–92 x 6–7 μ; Mexico.....v. *typicum*
Spores 55–74 x 5–7 μ, 7–11-septate; Colombia.....v. *granatense*
Spores 42–58 x 4–5 μ, 6–9-septate; Brasil.....v. *brasiliense*
Spores 36–50 x 7 μ, only 5-septate.....*C. leucocarpum*
Apothecia not white-pruinose.
Spores 48–66 x 6–8 μ; Colombia.....*C. implicatum*
Spores 38–50 x 6–8 μ; Chile.....*C. chilenum*
Spores 40–45 x 5–6.5 μ; apothecia sessile, margins turgid; Mexico.....*C. turgidulum*
Spores 30–37 x 2.5–3 μ; apothecial margins thin, blackening; Brasil.....*C. baculiferum*

COLLEMODIOPSIS Vainio, Étude Lich. Brésil 1: 234. 1890.
Type species: *Collema nigrescens* (Huds.) DC.

Spores slender, several-celled phragmospores, never muriform, apothecia with a pseudoparenchymatous cortex.

This subgenus is typically northern in its distribution. Only *C. lherminieri* Hue from Guadeloupe has been described previously from the American tropics.

Collema (Collemodiopsis) **Granadillae** Dodge, sp. nov.

Type: Costa Rica, Guanacaste, H. Granadilla, *Dodge & Thomas 6576*.

Thallus irregulariter subpinnatifide lobatus, lobis tenuibus, ad 1 mm. latis, viridi-nigricans, superne elevato-rugosus verrucosusque ad subisidiosus, inferne reticulatim elevato-rugosus, cinerascens, ad 400 μ crassitudine, decorticatus, rhizinis destitutus. Apothecium planum, peltatum, basi constrictum, 0.5–1.0 mm. diametro metiens, omnino nigrum, margine verrucosa; amphithecium 150–250 μ crassitudine, pseudoparenchymatice corticatum, intus algis nostocaceis subrectis; parathecium grosse pseudoparenchymaticum, bene evolutum, 100–120 μ crassitudine inferne, attenuatum ad 25–35 μ superne; hypothecium hyphis tenuibus dense contextum, 25–30 μ crassitudine; thecium 150–170 μ altitudine, paraphyses arcte cohaerentes septati, cylindrici, filiformes, ad 1 μ diametro metientes, clavato-capitati; ascosporae octonae, polystichae, rectae, fusiformes vel aciculares, 5-septatae, 37–42 x 4–5 μ.

Thallus irregularly subpinnately lobed, lobes slender, about 1 mm. broad, dark greenish black, with elevated folds and wrinkles above with masses of small subisidiose warts, below reticulately deeply scrobiculate wrinkled, more or less ashy, about 400 μ thick, without cortex or rhizoids. Apothecium peltate with constricted base, plane, about 0.5–1 mm. in diameter, wholly black, margin verrucose; amphithecium 150–250 μ thick, corticate with several layers of pseudoparenchyma below, within the filaments of *Nostoc* more or less straight and loosely interwoven; parathecium of large-celled pseudoparenchyma, about 100–120 μ thick below, thinning above at the margin to 25–35 μ; hypothecium of densely woven slender hyphae, 25–30 μ thick; thecium 150–170 μ tall; paraphyses closely adherent, septate, cylindrical, filiform, about 1 μ in diameter, clavate, capitate at the apex; ascospores 8 per ascus, polystichous, straight, fusiform or acicular, 6-celled, 37–42 x 4–5 μ.

Guanacaste: H. Granadilla, *Dodge & Thomas 6576;* near Tilarán, *Standley & J. Valerio 44529,* 640–660 m., *Dodge & Thomas 6560.*

COLLEMA LEUCOPEPLA (Tuck.) Schneider, Guide Study Lich., 181. 1898.

Collema nigrescens var. *leucopepla* Tuck., Syn. N. Am. Lich. 1: 148. 1882.

Type: not stated in original description, based on material from South Carolina, Georgia, Florida, Alabama, and Louisiana.

It is probable that material collected in Puntarenas, Boruca, 560 m., *Tonduz 5373,* and determined by Müller Argau as *C. nigrescens* var. *caesium* Ach., belongs here but I did not have time to study it critically while I was in Genève. This species of the lower Atlantic and Gulf Coastal Plain in the United States has also been reported by Vainio from Mexico.

DICOLLEMA (Clements) Dodge, n. subgenus.

Dicollema Clements, Gen. Fung. 74. 1909.

Type species: *Collema pycnocarpum* Nyl.

Thallus narrowly laciniate or incised, spores clavate to fusiform, typically 2-celled, rarely 4-celled.

The material referred to the type species of this genus by different authors is quite variable and the whole group needs a thorough revision. Pending such a study, I am proposing several varieties of *C. pycnocarpum* Nyl. to cover the more conspicuous variations which seem to have separate geographical ranges, those with the smaller spore sizes being rather more southern in their distribution.

COLLEMA PYCNOCARPUM Nyl., Syn. Meth. Lich. 1: 115. 1858.

Type: United States [on journey from New York to Philadelphia], *Moré.*

Thallus dark or pale green, medium in size, granulate-nodose. Apothecia rufous, crowded, almost contiguous, at first plane, then somewhat convex, quite small, 0.5 mm. or a little larger; spores 8 per ascus, oblong or oblong-ellipsoid, simple or uniseptate, 14–17 x 4–5 μ.

Northern Atlantic Coastal Plain.

Var. **Minarum** Dodge, var. nov.

Collema pycnocarpum Vainio, Étude Lich. Brésil 1: 238. 1890.

Type: Brasil, Minas Geraes, Sitio, 1000 m., *Vainio 734.*

Sporae oblongae, ellipsoideae vel fusiformes, 1-septatae, 6–9 x 3 μ.

Var. **Malmei** Dodge, var. nov.

C. pycnocarpum Malme, Ark. f. Bot. **19**[8]: 7. 1924.

Type: Brasil, Rio Grande do Sul, Canõas near Porto Alegre, *Malme 536*.

Sporae oblongo-ellipsoideae vel ellipsoideae, uniseptatae, 10–14 x 3–4 μ.

Var. **crassiusculum** (Malme) Dodge, comb. nov.

Forma *crassiusculum* Malme, Ark. f. Bot. **19**[8]: 7. 1924.

Collema pycnocarpum Nyl., Acta Soc. Sci. Fenn. **7**: 428. 1863.

Type: Brasil, Matto Grosso, Corumbà, *Malme*.

Sporae ellipsoideae, utroque apice obtusae, raro acutae vel altero obtusae, altero acutae, 10–15.5 x 4–5.5 μ.

The specimens cited by Nylander from Colombia, Bogotá, 2600 m., *Lindig 2872*, have spores 10–14 x 4.5–5.5 μ.

Collema cyrtaspis Tuck., Proc. Amer. Acad. Arts & Sci. **5**: 387. 1862.

Type: no type mentioned in the original description.

Thallus deeply and irregularly lobed, verrucose, deep blue green, darkening on drying, verrucae much larger than in *C. pycnocarpum*, about 400 μ thick; algal layer about 100 μ thick, composed of coiled and tangled filaments of *Nostoc* imbedded in a gel, cells spherical, about 4 μ in diameter; medulla gelified, traversed by very loosely tangled hyphae about 2–3 μ in diameter and occasional straight filaments of *Nostoc*. Apothecia immersed in the verrucae when young, becoming plane or even convex, up to 2 mm. in diameter, margin slightly verrucose when young, practically disappearing as the apothecium becomes increasingly complex; amphithecium about 160 μ broad, of the same texture as the thallus; parathecium lacking; hypothecium filamentous, quite highly developed, about 40 μ thick, not conspicuously thinner toward the margins, hyphae 2–3 μ in diameter, very loosely woven; thecium about 80 μ tall; paraphyses filamentous, clavate, expanded above, imbedded in a gel; asci clavate, about 40 x 8 μ, 8-spored; ascospores subfusiform, 2–4-locular, 16–25 x 3–7 μ [16–20 x 4–4.5 μ in Costa Rican specimens].

This species is widely distributed in the southern Atlantic

and Gulf Coastal Plain in the United States, extending up the Mississippi Valley to Iowa.

Guanacaste: Liberia, 100 m., *Dodge & Thomas 8016, 8027.*

SYNECHOBLASTUS (Trevis.) Vainio, Étude Lich. Brésil **1**: 234. 1890.

Lathargium S. F. Gray, Nat. Arrang. Brit. Pl. **1**: 399. 1821, p. p.

Synechoblastus Trevis., Caratt. Tre Nuov. Gen. Coll. 3. 1853, p. p.

Type species: as subgenus based on *C. glaucophthalmum* Nyl.

Apothecia without pseudoparenchymatous cortex, spores fusiform to more or less acicular, many-celled, mostly over 30 μ long.

This subgenus is typically northern. Only two species are known from Costa Rica, where they are temperate species at elevations between 1000 and 1800 m., and are also found in the highlands of Colombia at somewhat greater altitudes.

COLLEMA GLAUCOPHTHALMUM Nyl., Syn. Meth. Lich. **1**: 114, 115. 1858.

Type: Mexico, Orizaba, *Fr. Müller.*

Thallus olivaceous-fuscous, medium size, expanded, more or less fenestrate and dissected, scrobiculate, and often granuliferous. Apothecia glauco-lilac colored, plane and somewhat concave, margin prominent, thin; ascospores 77–92 x 6–7 μ.

Var. GRANATENSE Hue, Jour. de Bot. [Morot] **20**: 12. 1906.

Collema glaucophthalmum Nyl., Acta Soc. Sci. Fenn. **7**: 428. 1863, non loco alio.

Type: Colombia, Choachí, 2600 m., *Lindig 813.*

Thallus dusky yellowish green to almost black, irregularly lobed and fenestrate, smooth or somewhat scrobiculate, coarsely wrinkled and subverrucose, especially toward the tips, margins thick and rounded, 800–1000 μ thick, homoeomerous, of loosely tangled filaments of *Nostoc*, cells 5–6 x 2.5–3 μ, ellipsoidal. Apothecia peltate, constricted at the base, up to 3 mm. in diameter, margin at first prominent, smooth, becoming thinner, less prominent and verrucose at maturity; disc chalky white to vinaceous-russet; amphithecium about 160 μ thick, homogeneous with the thallus; parathecium pseudoparenchymatous, 40 μ thick, thinning to 20 μ or even disappearing above at the margin;

hypothecium filamentous, of large very densely interwoven hyphae about 40 μ thick; thecium about 120 μ tall; paraphyses filiform, about 1 μ in diameter, not swollen above; asci clavate, about 20 μ in diameter, 8-spored; ascospores polystichous, fusiform to acicular, 55–74 x 5–7 μ.

The whiteness of the apothecial disc is very variable on the same thallus, in general being more pronounced on young and rapidly growing apothecia and gradually disappearing on older and more exposed ones. It seems to be a semi-crystalline deposit which slowly dissolves away, disclosing the vinaceous-russet disc formed by the discoloration of the upper portion of the gel surrounding the paraphyses. This variety has previously been reported only from Colombia.

Cartago: near R. Birrís above Santiago, 920–1340 m., *Dodge 8011*, *Dodge & Thomas 8015;* Carpintera, 1700 m., *K. Danielson 102.*

San José: Sta. María de Dota, 1500–1800 m., *Standley & J. Valerio 44150.*

COLLEMA IMPLICATUM Nyl., Acta Soc. Sci. Fenn. **7**: 428. 1863.

Type: Colombia, Villeta, 1100 m., *Lindig 749;* Cundinamarca, Bogotá, 2400–2600 m., *Lindig.*

Thallus dark greenish black, broadly lobed and fenestrate, smooth near margins, becoming deeply reticulate-scrobiculate in older portions of the thallus, verrucae scattered, more elevated, often subisidioid, margins thin and semipellucid, homoeomerous, of loosely tangled filaments of *Nostoc*, cells 4 x 5 μ, heterocysts 5 x 8 μ, much more closely tangled in an outer zone about 150 μ thick. Apothecia peltate, constricted at the base, up to 4 mm. in diameter, margin at first prominent, verrucose, becoming thinner, less prominent, scarcely visible as the apothecium becomes expanded and convex, disc cameo-brown, without pruina in young apothecia; amphithecium about 100 μ thick, homogeneous with the thallus; parathecium pseudoparenchymatous, about 80 μ thick below, thinning out to a single layer of thick-walled cells 6 μ wide above at the margin; hypothecium of densely woven hyphae about 20 μ thick; thecium about 100 μ tall; paraphyses about 2 μ in diameter, with clavate brown tips; asci clavate, 8-spored, 12–16 μ in diameter; ascospores polystichous, fusiform to acicular, 48–66 x 6–8 μ, 7–9-septate.

This species is very closely related to *C. glaucophthalmum*

and has been reduced to synonymy by some authors. It differs from the previous species in several minor details of proportion of measurements and in the lack of a well-developed pruina. It is to be hoped that some one will be able to study the two forms in the field to determine the constancy of this character. In a series of specimens one can find considerable variation in the amount of pruina in *C. glaucophthalmum*. It should be noted that the two species occupy the same areas in Costa Rica.

Cartago: above R. Birrís at Santiago, 1140–1180 m., *Dodge 8017;* Carpintera, 1700 m., *K. Danielson 101c.*

The following species has not been found in Costa Rica but it is closely related to this group.

COLLEMA (SYNECHOBLASTUS) **Ramboi** Dodge, sp. nov.

Type: Brasil, Rio Grande do Sul, Porto Alegre, *B. Rambo 74.*

Thallus parvus, adscendens, obscure viridi-nigricans, angustilobatus, fenestratus clathratusque, verrucis elevatis isidioideis, marginibus plus minusve integris, tenuibus, pellucidis, 130–140 μ crassitudine, homoeomerus, filamentis nostocaceis laxe implexis, in zona exteriori 40 μ crassitudine dense contextis, cellulis ad 3 x 6 μ. Apothecium peltatum, basi constrictum, planum, 0.5–0.6 (– 1.0) mm. diametro, margine tenui, verrucosa subcrenulatave, disco obscure castaneo nigroque; amphithecium 20 μ crassitudine, cum thallo homogeneum; parathecium deest; hypothecium 20 μ crassitudine, hyphis tenuibus dense contextum; thecium 160 μ altitudine; paraphyses filiformes, ad 1 μ diametro, apicibus inflatis; asci clavati, 120 x 8–10 μ; ascosporae octonae, polystichae, aciculares, immaturae, plus quam 100 μ longitudine, multiloculares.

Thallus small, elevated, dark greenish black, narrowly lobed and fenestrate, verrucae elevated and isidioid, margins more or less smooth, thin, pellucid, 130–140 μ thick, homoeomerous, of loosely tangled filaments of *Nostoc*, cells about 3 x 6 μ, much more closely tangled in an outer zone about 40 μ thick. Apothecia peltate, constricted at the base, 0.5–0.6 (– 1.0) mm. in diameter, plane, margin very thin, verrucose, subcrenulate, finally almost disappearing while the expanded apothecium remains plane, disc dark chestnut to black; amphithecium 20 μ broad, homogeneous with the thallus; parathecium absent; hypothecium 20 μ

thick, of slender densely woven hyphae; thecium about 160 μ tall; paraphyses filiform, about 1 μ in diameter with greatly swollen tips, imbedded in a hymenial gel; asci clavate, 8-spored, 120 μ long, 8–10 μ wide; ascospores polystichous, 8 per ascus, acicular, immature and very difficult to measure but somewhat more than 100 μ long, many-celled.

LEPTOGIUM S. F. Gray

LEPTOGIUM S. F. Gray, Nat. Arrang. Brit. Pl. 1: 400. 1821.
Type species: *Leptogium tremelloides* S. F. Gray, excl. syn.

Thallus foliose in the tropical species, gelified, below naked (covered with rhizinae in section *Mallotium*), with pseudoparenchymatous cortex above and below, algae *Nostoc*. Apothecia sunken in the thallus at first, emerging and often short-stipitate; amphithecium often corticate with several layers of pseudoparenchyma below, usually of a single layer above; parathecium usually present and pseudoparenchymatous, often thinning out above at the margin, sometimes of large, more or less parallel hyphae; paraphyses simple and filiform, often with apex variously thickened; asci clavate to cylindrical, 8-spored; ascospores usually imbricately monostichous, occasionally distichous, hyaline, usually broadly fusiform with acute to acuminate ends (acicular in section *Leptogiopsis*), usually muriform (except in *Leptogiopsis*), with thin walls.

Four of the seven sections into which *Leptogium* is divided are found in tropical America, all four of them in Costa Rica. *Leptogiopsis* differs from all the other sections in its acicular, never muriform spores, and perhaps should be treated as a separate genus, more or less related to section *Synechoblastus* of *Collema*. It is endemic in tropical America, with a single unidentifiable fragment yet found in Costa Rica. *Mallotium*, to which may also be referred the section *Leptolobaria* of Vainio based on *L. callithamnium* from Chile and the Antilles, has highly developed rhizinae below, and often more or less tomentum above, especially in the vicinity of the apothecia. It is widespread in its distribution, being found in the temperate zones and evidently preferring the colder regions of the tropics, not occurring below about 1300 m., in Costa Rica not seen below 3000 m. *Diplothallus*, with

its two separate layers of thallus, each with its own cortex, connected by pillars of pseudoparenchyma which on drying cause punctate depressions in the thallus, is endemic in tropical America and confined to the temperate regions, in Costa Rica occurring between 1200 and 1700 m., coming down to lower levels in the mountain near H. Santamaría of the Cordillera de Tilarán in Guanacaste. *Euleptogium*, which contains the most species, is found from sea level to about 1800 m. in Costa Rica, although most of the species of this section have a narrower altitudinal range. The lowland species ascend the river valleys but mostly drop out at about 1000 m. These are usually widespread at low elevations, extending from the Atlantic and Gulf Coastal plains of the United States to southern Brasil and Paraguay. The species of the temperate region of Costa Rica are mostly confined to the mountains from southern Mexico to the northern Andes and the mountains of eastern Brasil.

The morphology of the apothecium is not altogether clear and considerable confusion exists in the nomenclature of the various parts. In the following discussion, I have treated the tissue, usually well differentiated, underlying the hypothecium, as the parathecium, whether it extends to the surface of the thecium or not and whether or not it is pseudoparenchymatous. In a very few species the parathecium as thus defined thins out and disappears near the edge of the thecium, and the hyphae of the hypothecium extend up to the surface of the thecium. It is possible that some would prefer to regard the parathecium as formed of two layers, the lower of which is pseudoparenchymatous and the upper filamentous, with only the upper extending to the surface of the thecium. The amphithecium (thalline margin of earlier lichenologists) is essentially homogeneous and continuous with the thallus, although the algae are sometimes more densely tangled and in many species the cortical cells on the under side of the amphithecium (morphologically continuous with the upper surface of the thallus) or even the under side of the thallus below the apothecium, may proliferate, forming a pseudoparenchyma of several layers of cells sometimes even thicker than the algal zone.

KEY TO THE TROPICAL AMERICAN SPECIES OF LEPTOGIUM

Spores acicular, never muriform.
- Thallus reticulate-scrobiculate; French Guiana. [Spores 6-celled, 35 x 10 μ, teste Leighton, in specimen from Amazonas, Brasil] ..... *L. reticulatum*
- Thallus not reticulate-scrobiculate.
  - Spores 48 μ long, 8–10-celled; Florida ..... *L. fusisporum* (Tuck.) Dodge
  - Spores 50–60 x 7–8 μ, 10–12-celled; Mexico ..... *L. adpressum*
  - Spores 65–85 x 3.5–5 μ, 10–12-celled; Brasil ..... *L. megapotamicum*

Spores muriform.
- Thallus of two lamellae, each with its own cortex, attached to each other by columns of tissue ..... DIPLOTHALLUS
  - A single species ..... *L. diaphanum*
- Thallus of a single lamella, not as above.
  - Thallus with a thick tomentum of rhizinae below ..... MALLOTIUM
    - Apothecia on lower surface, lobes of thallus broad, imbricate .... *L. resupinans*
    - Apothecia on upper surface.
      - Upper surface of thallus tomentose, at least near apothecia.
        - Margins of sinuses inrolled, lobes medium, sinuate; apothecial margins microphylline ..... *L. inflexum*
        - Margins of thallus isidiose dissected.
          - Upper surface of thallus subglabrous and more or less granular-papillate ..... *L. papillosum*
          - Upper surface of the thallus arachnoid-tomentose, lobes minutely dissected, not otherwise isidiose; Chile. *L. callithamnium*
          - Upper surface of thallus glabrous except in the vicinity of the apothecia; apothecial margin isidiose, scattered groups of isidia sometimes on the upper surface of the thallus. ..... *L. inflexum* v. *isidiosulum*
  - Thallus corticate with a single layer of cells on both surfaces, without rhizinae ..... EULEPTOGIUM
    - Thallus scrobiculate.
      - Margin of thallus papillate-denticulate, olivaceous, thallus deeply reticulate-scrobiculate; parathecium filamentous; spores 22–24 x 12–14 μ ..... *L. olivaceum*
      - Margin of thallus smooth, thallus plumbeous.
        - Thallus deeply reticulate-scrobiculate; spores 30–40 x 12–16 μ. ..... *L. foveolatum*
        - Thallus shallowly scrobiculate; spores 20–30 x 8–10 μ... *L. microstictum*
    - Thallus variously wrinkled on drying, but not scrobiculate, usually nearly smooth when moist.
      - Apothecia on tips of long swollen processes of the thallus.
        - Thallus lobes narrow, dichotomously branched, wrinkles irregular ..... *L. stipitatum*
        - Thallus lobes broader, not conspicuously dichotomously branched, wrinkles predominantly longitudinal or radial, secondary wrinkles mostly periclinal to margins.
          - Apothecia radially wrinkled to smooth; spores 23–32 x 10–13 μ ..... *L. vesiculosum*
          - Apothecia with periclinal wrinkles, foliolate or isidiose.
            - Thallus and apothecia not isidiose.

Apothecia small, spores 22–30 x 10–12 μ, margin wrinkled.
Thallus lobes broad.....*L. phyllocarpum*
Thallus lobes narrower, margins crisped.....v. *campestre*
Apothecia rather large with more or less foliolate margins.
.....v. *macrocarpum*
Thallus and apothecia isidiose, often densely so; spores 30–40(–45) x 12–17 μ.....*L. coralloideum*
Apothecia marginal, sessile or on short solid stalks, very minute.
Thallus dark green to black, exciple isidiose [spores 24–30 (–33) x 12–14 μ, transverse septa 5, fide Malme]; Jamaica.....*L. chloromelan*
Thallus ashy to plumbeous.
Exciple isidiose, parathecium 150 μ thick; thecium 150–180 μ tall.....*L. marginellum*
Exciple verruculose, parathecium 100 μ thick; thecium 120–140 μ tall; Paraguay.....*L. pilcomayense*
Exciple crenulate, parathecium 30 μ thick; thecium 110–130 μ tall; Paraguay.....*L. microcarpum*
Apothecia scattered on upper surface of the thallus.
Thallus thick, 250 μ or more, roughened below by more or less conical outgrowths but rhizinae absent.
Smooth above or slightly wrinkled, lobes ascending, obovate; Mexico.....*L. hypotrachynum*
Reticulately wrinkled above, lobes appressed, short, wide, margins sometimes crisped; Brasil.....*L. mattogrossense*
Smooth or only slightly wrinkled below.....*L. sessile*
Thallus thinner, seldom reaching 200 μ.
Thallus more or less isidiose.
Isidia blackening, cylindric; parathecium well developed.
Spores 18–23 x 8–10 μ, transverse septa 3, rarely 5; Brasil and Paraguay.....*L. pichneum* Malme excl. syn.
Spores 22–30 x 11–13 μ, transverse septa 5.....
.....*L. simplicius* v. *pichneoides*
Isidia concolorous, parathecium not well developed.....
.....*L. denticulatum* Nyl.
Thallus 100 μ thick; apothecia not isidiose; spores 22–27 x 7–9 μ, transverse septa usually 5; isidia coralloid branched or somewhat flattened; Brasil.
.....*L. austroamericanum* (Malme) Dodge
Thallus about 30 μ thick; apothecial margins verrucose, spores 16–23 x 8–9 μ, transverse septa 3; isidia often flattened and microphylline.....
.....*L. denticulatum* Malme non Nyl.
Isidia of hemispheric concolorous granules, thallus rigid, irregularly undulate, wrinkled toward the margin; Paraguay.....*L. granulare*
Thallus not isidiose.
Parathecium filamentous, or, if pseudoparenchymatous, very thin and inconspicuous.

Cortex of amphithecium a single layer of cells; thallus 50–75 μ thick; apothecia rarely 1 mm. in diam.; spores 15–19 x 6–8 μ with 3 transverse septa; Brasil.....*L. Puiggarii*

Cortex of amphithecium a single layer of cells above, becoming thick, pseudoparenchymatous below; apothecia 1–3 mm. in diam.

Thallus greenish black, 80–100 μ thick; spores 15–22 x 6–8 μ, with 3–5 transverse septa; Brasil...*L. brasiliense*

Thallus ashy, plumbeous, or bluish.

Thallus papulose and wrinkled above, opaque, 130–160 μ thick; spores 20–30 x 7–12 μ..*L. pulchellum*

Thallus minutely wrinkled above, opaque, 150–170 μ thick; spores 24–28 x 12 μ.....*L. Standleyi*

Thallus minutely wrinkled above, margins inrolled; spores 38–46 x 16–18 μ.....*L. dimorphum*

Thallus smooth or minutely wrinkled on drying, pellucid, less than 100 μ thick.

Spores 22–28 x 8–10 μ; thecium 120–150 μ tall; thallus 80–100 μ thick.....*L. azureum*

Spores 18–22 x 10–12 μ, with 3–5 transverse septa; thecium 90–120 μ tall; thallus 35–60 μ thick.....*L. Tuckermani*

Spores 18–24 x 8–9 μ with 3–5 transverse septa; thecium 140–160 μ tall; thallus 35–50 μ thick; Brasil.....*L. Schiffneri*

Parathecium pseudoparenchymatous.

Spores 35–40 x 16–20 μ; thallus plumbeous to greenish; amphithecium smooth; Bolivia.....*L. laevius* (Nyl.) Dodge

Spores 30–40 x 12–17 μ; thallus rather dark plumbeous to black.

Laciniae 1–1.5 mm. broad, apothecia not over 1.5 mm. in diam.; thecium 170 μ tall; paraphyses 1.5–2 μ thick; Brasil.....*L. Lafayetteanum*

Laciniae up to 8 mm. broad, apothecia 1–2.5 mm. in diam.; thecium 190–220 μ tall; paraphyses 2.5–3 μ thick; Brasil.....*L. pachycheilum*

Spores 25–35 x 12–15 μ; apothecia 1–2.5 mm. in diam.; thecium 150–180 μ tall; paraphyses 2–2.5 μ thick; Brasil.....*L. ulvaceum* Malme non Pers.

Spores 22–30 x 11–13 μ; apothecia 1.5–2.2 mm. in diam.; thecium 150–170 μ tall.....*L. simplicius*

Spores 18–34 x 9–15 μ; thallus dark green to plumbeous; amphithecium granulate to wrinkled.

Thalline lobes ascendant, gyrose-plicate..*L. conchatum* (Tuck.) Dodge (*L. chloromelan* auct. non Sw.)

Thalline lobes narrower, edges erect, crisped..*L. stellans* (Tuck.) Dodge

DIPLOTHALLUS Vainio, Étude Lich. Brésil 1: 222. 1890.

Thallus of two lamellae connected by pillars. Each lamella

has a cortex of a single layer of isodiametric cells above and below, drying impressed-punctate. Rhizinae absent, spores muriform.

LEPTOGIUM DIAPHANUM (Sw.) Mont., Ann. Sci. Nat. Bot. III, 10: 134. 1848.

Lichen diaphanum Sw., Nov. Gen. Sp. Pl. Prodr. 147. 1788.

Parmelia diaphana Ach., Meth. Lich. 223. 1803.

Collema diaphanum Ach., Lichenog. Univ. 654. 1810.

Leptogium punctulatum Nyl. ap. Fournier, Mexic. Pl. 1: 1. 1872.

Leptogium tremelloides var. *impressopunctatum* Tuck. ap. Williams, Amer. Nat. 29: 482. 1895.

Type: Jamaica, *Swartz*, in Riksmuseet, Stockholm, carefully described by Malme, Ark. f. Bot. 19[8]: 27. 1924.

Thallus mineral gray, lobes rounded, ascending, crowded, impressed-punctate, otherwise quite smooth, consisting of two layers, each about 40 μ thick, composed of a row of pseudoparenchymatous cells about 8 μ in diameter on each surface and an algal layer of *Nostoc* between, the layers connected by pseudoparenchymatous pillars whose contraction in drying form the depressions. Apothecia borne on the upper layer, peltate, constricted at the base, 1–1.5 mm. in diameter, margin light buff, smooth, disc chestnut; amphithecium corticate with thick pseudoparenchyma below, about 80 μ thick, thinning to two layers of cells above next the disc, algal layer about 30 μ thick below the thecium; parathecium absent; hypothecium of densely woven, large, thick-walled hyphae about 50 μ thick; thecium 120–130 μ tall; paraphyses filiform, 1–2 μ thick, with clavate tips, forming a brown epithecium imbedded in a gel; asci clavate to cylindric, about 12–16 μ in diameter, thin-walled tips thickened and staining deep blue with iodine; spores 8 per ascus, imbricately monostichous, fusiform, muriform, 16–25 x 7–8 μ, with 3–5 transverse septa.

This species is found in the mountains from Mexico and the West Indies, Dominican Republic, and Jamaica (900–1200 m.) to Minas Geraes (1400–1500) m., Bolivia (1900 m.), and Perú (1700 m). In Costa Rica it is found from 1200 to 1700 m. and descends to 800 m. on the mountain back of the farmhouse at

H. Santamaría where the peculiar weather conditions enable many species to flourish below their normal altitudes. Apparently it needs high humidities as it is found mostly in localities of frequent and long-continued fogs (see p. 379).

Cartago: R. Birrís above Santiago, 1220–1340 m., *Dodge & Thomas 7938;* Cartago, *R. Torres R. 143;* Carpintera, 1700 m., *K. Danielson 103.*

San José: Sta. María de Dota, 1500–1800 m., *Standley & J. Valerio 43208.*

Heredia: C. Central de Zurquí, 1600–1700 m., *Dodge, J. Valerio & Thomas 4824.*

Guanacaste: H. Santamaría, 720–850 m., *Dodge, Jiménez & Thomas 7017.*

MALLOTIUM Ach., Lich. Univ. 644. 1810.

Mallotium Gray, Nat. Arrang. Brit. Pl. **1**: 399. 1821.

Type: *Collema saturninum* (Dicks.) Ach.

Thallus foliose, cortex of pseudoparenchyma above, below tomentose with rhizinae; spores muriform.

This subgenus, considered as a separate genus by many authors, is cosmopolitan in distribution, mostly along cold foggy coasts or in mountains, being abundant in species in northern Europe and in Patagonia, Tierra del Fuego, and Antarctic Islands. Our tropical species seem confined to much higher elevations (above 1800 m. in Costa Rica) than the other members of *Leptogium.* It is quite distinct in appearance, often tomentose above, so that sterile specimens might be taken for *Erioderma* or *Umbilicaria* on macroscopic examination.

Our two species, *L. inflexum* and *L. papillosum*, are both Mexican, the former extending to Perú, the latter not known south of Costa Rica.

LEPTOGIUM **papillosum** (Bouly de Lesdain) Dodge, comb. nov.

Leptogium Hildenbrandii var. *papillosum* Bouly de Lesdain, Lich. Mexique, 30. 1914.

Type: Mexico, Michoacan, Puebla, H. Batan, *G. Arsène Brouard 4212;* Morelia, C. Azul, *G. Arsène Brouard 3999.*

Thallus mineral gray above, dark olive buff below, lobes subpinnatifid, with small, lacerate, almost isidioid margins, papillate-granulose, sometimes isidiose above, densely tomentose below, algal layer about 60 μ thick, corticate on each surface, with cells 5–6 μ in diameter, the cortex of the lower surface giving rise to a dense covering of rhizinae. Apothecia submarginal but too immature in our specimens to show details of structure well.

San José: L. de la Chonta, n. e. Sta. María de Dota, 2000–2100 m., *Standley 42285;* near Sta. María de Dota, 1500–1800 m., *Standley 41643.*

LEPTOGIUM INFLEXUM Nyl., Flora **41**: 377. 1858; Syn. Meth. Lich. **1**: 132. 1858.

Type: Mexico, Orizaba, *Fr. Müller.*

Thallus deep glaucous gray, dark olive buff below, lobes irregular, rounded, margins crisped and subascending, smooth above except in the vicinity of the apothecia, densely tomentose below, algal layer about 60 μ thick, corticate on both sides with two layers of cells about 8–10 μ in diameter, the lower surface giving rise to rhizinae, as also the outer cortical cells in the vicinity of the apothecia. Apothecia large, up to 4 mm. in diameter, margin more or less tomentose below, phyllophorous, concolorous with the thallus, disc orange rufous to auburn, edge of parathecium lighter; amphithecium with pseudoparenchymatous cortex about 100 μ thick, thinning to about one or two cells thick above, algal layer of *Nostoc* about 80 μ thick; parathecium pseudoparenchymatous, about 20 μ thick below the hypothecium, expanding upward to thickness of 100 μ above; hypothecium 30 μ thick, of densely woven hyphae; thecium 240 μ tall; paraphyses filiform, 1–2 μ in diameter, with swollen clavate tips forming a brown epithecium; asci 20–25 μ in diameter, cylindrical; ascospores monostichous, 8 per ascus, broad, fusiform, muriform, with 7 transverse septa, about 35–40 x 13–17 μ.

Superficially the thallus suggests the texture of *L. tremelloides,* while the apothecia might easily be mistaken for *L. phyllocarpum,* although the rhizinae below should be easily observed.

Cartago: F. Volcán de Turrialba, 2000–2400 m., *Standley 34966, 35174.*

San José: Quebradillas, 7 km. n. Sta. María de Dota, *Standley 42899;* C. de las Vueltas, 2700–3000 m., *Standley & J. Valerio 43823a.*

Var. ISIDIOSULUM Nyl., Acta Soc. Sci. Fenn. **7**: 429. 1863; Ann. Sci. Nat. Bot. IV, **19**: 289. 1863.

Type: Colombia, Paramo Choachí, 3600 m., *Lindig.*

This variety differs in the thallus being partly isidio-furfuraceous, especially the margins of the lobes; apothecial margin isidiose instead of phyllophorous, subnude below; spores 36–44 x 18–25 μ.

This variety would seem to differ from *L. papillosum* in that the isidia are in scattered groups or marginal, much more highly

developed and more denuded below. A single sterile specimen has been referred here.

San José: C. Zurquí, 2000–2500 m., *Standley & J. Valerio 48292.*

EULEPTOGIUM Tuck., Gen. Lich. 95. 1872.

Stephanophorus Fw., Linnaea 17: 29. 1843.

Type species: no species cited.

Thallus foliose, monophyllous, without rhizinae, upper and lower cortex present, each of a single layer of isodiametric cells; spores muriform, many-celled.

This subgenus is predominantly tropical with many species in tropical America.

LEPTOGIUM OLIVACEUM (Hook.) Zahlbr., Cat. Lich. Univ. 3: 146. 1924, excl. syn.

Collema olivaceum Hook. ap. Kunth, Syn. Pl. Aequinoct. Orb. Nov. 1: 38. 1822.

Type: Colombia, Cauca, between Popayan and Almaguer, *Humboldt 252.*

Thallus isabella to light brownish olive, lobes broad and rounded, surface deeply reticulate-scrobiculate with sharp ridges and margins granulate-isidiose, or ridges rarely microphylline; about 200 μ thick, corticate above and below with an algal layer of loosely tangled filaments of *Nostoc* corresponding to a medulla, separated from the cortex by a palisade layer about 30 μ thick. Apothecia sessile, margin verrucose, concolorous, disc darker; amphithecium thick, homogeneous with the thallus; parathecium about 80 μ thick, of very densely woven hyphae; hypothecium about 25 μ thick, very deeply staining; thecium about 100 μ tall; paraphyses filiform, about 1.5 μ in diameter, ending in the epithecial gel.

The apothecia are very rare and too immature in the one specimen from Costa Rica to give all the characters, but it seems quite distinct from *L. reticulatum* and *L. foveolatum.*

Cartago: R. Reventazón below Santiago, 740–750 m., *Dodge 8010.*

Var. **granulosum** Dodge, var. nov.

Type: Costa Rica, San José, R. Virilla below El Brazil, *Dodge 8030.*

Thallus similis speciei sed nigro-granulatum ambobus superficiebus. Apothecia obscure brunnea, nigricantia, sessilia, basi

constricta, disco concolore; amphithecium 190 μ crassitudine, corticatum cellulis isodiametricis; parathecium 130–140 μ crassitudine, ad marginem 40 μ attenuatus, hyphis dense contextum; hypothecium 30 μ crassitudine; thecium 120–130 μ altum; paraphyses similes speciei; asci 12–16 μ diametro metientes; ascosporae imbricatim monostichae, octonae, fusiformes, 22–24 x 12–14 μ.

Thallus similar to that of the species but black-granulate on both surfaces. Apothecia dark brown, blackening, sessile, constricted at the base, disc concolorous; amphithecium 190 μ thick, homogeneous with the thallus, corticate with a single layer of isodiametric cells; parathecium 130–140 μ thick, thinning toward the margin to 40 μ, of densely woven hyphae; hypothecium 30 μ thick, so deeply staining that its structure is not clear; thecium 120–130 μ tall; paraphyses as in the species; asci cylindric, 12–16 μ in diameter; ascospores imbricately monostichous, 8 per ascus, fusiform, 22–24 x 12–14 μ.

San José: R. Virilla below El Brazil, *Dodge 8030*.

LEPTOGIUM FOVEOLATUM Nyl., Syn. Meth. Lich. 1: 124. 1858.

Type: not stated, specimens from Bolivia, *Weddell*, and Mexico, *Fr. Müller*, are mentioned.

Thallus mineral gray or darker, often discolored light yellowish olive or darker, lobes rounded, smooth, surface deeply foveolate with sharp wrinkles both above and below; very variable in thickness up to 250 μ, *Nostoc* very sparsely scattered throughout, rather denser and tending to form a palisade layer just below the surface, but this layer much thinner and less definite than in *L. olivaceum*. Apothecia about 1.5 mm. in diameter, constricted at the base, margin smooth, light buff, disc cinnamon rufous; amphithecium about 130 μ thick, similar to the thallus in structure but algae much more abundant and closely tangled and cortex pseudoparenchymatous, about 50 μ thick; parathecium about 40 μ thick below, thinning out and disappearing above, pseudoparenchymatous with small cells; hypothecium about 80 μ thick, very deeply staining; thecium about 170 μ tall; paraphyses filiform, about 2 μ in diameter, tips clavate; asci 8-spored, cylindrical, about 12 μ in diameter; ascospores imbricately

monostichous, 30–40 x 12–16 μ [only 20 x 10 μ in Costa Rican specimens, but obviously immature].

San José: R. Virilla below El Brazil, *Dodge 7783.*

LEPTOGIUM MICROSTICTUM Vainio, Dansk Bot. Ark. 4[11]: 18. 1926.

Type: not stated, specimens cited, Mexico, Palenque, *Liebmann 7415, 7416, 7428;* Papantla, *Liebmann.*

Thallus light glaucous blue or a little darker, lobes rounded, surface smooth but irregularly impressed, margin thin and smooth; about 225 μ thick, corticate on both surfaces with cells about 4 μ in diameter, the filaments of *Nostoc* very loosely tangled, more or less parallel to the surface and very scanty in the middle. Apothecia 0.5–1.0(– 1.3) mm. in diameter, sessile, constricted at the base, margin thin, whole, concolorous with the thallus, disc pale to tawny; amphithecium about 60 μ thick, cortex pseudoparenchymatous, about 25 μ thick below, thinning out above to a double layer of cells, algal layer of tangled filaments of *Nostoc;* parathecium pseudoparenchymatous, about 40 μ thick, thinning above to about three layers of cells; hypothecium 30 μ thick, of densely tangled rather large hyphae suggesting pseudoparenchyma but cells much smaller than in the parathecium; thecium about 100 μ tall; paraphyses filiform, 1 μ in diameter, ending in the epithecial gel; asci clavate to cylindrical, 12–16 μ in diameter; ascospores distichous, 8 per ascus, fusiform, with about 5 transverse septa, 20–30 x 8–10 μ.

A single small specimen from Costa Rica is doubtfully referred here. The thallus is somewhat more finely impressed and occasionally near the margin it appears very minutely scrobiculate. This specimen is from a greater elevation than most other members of this subgenus.

San José: Guayabillos and Cabeza de Vaca, 2150–2350 m., *Dodge & Thomas 7418.*

LEPTOGIUM VESICULOSUM (Sw.) Malme, Ark. f. Bot. 19[8]: 14–15. 1924.

Lichen vesiculosus Sw., Nov. Gen. Sp. Pl. Prodr. 147. 1788.

Lichen bullatum Ach., Lichenog. Suec. Prodr. 137. 1798.

Leptogium bullatum Mont., Ann. Sci. Nat. Bot. II, 16: 113. 1841.

Type: Jamaica, tops of mountains, *O. Swartz. L. bullatum* Ach. based on the same material.

Thallus plumbeous, lobes rounded, more or less appressed, longitudinally wrinkled with shallower cross ridges; about 100 μ thick between the ridges, corticate on each surface with a single layer of isodiametric cells, homoeomerous, of very loosely tangled filaments of *Nostoc* running more or less parallel to the surface. Apothecia sunk in the tips of podetiiform elevations of the thallus, margin wrinkled but not isidioid or phyllophorous, edge of parathecium conspicuous light buff, disc concave, rufous; amphithecium continuous with the thallus without differentiation, cortex a single layer of cells both above and below; parathecium 100 μ thick below, thinning out to 40–60 μ above at the margin, pseudoparenchymatous; hypothecium thick, 80 μ, of densely woven slender hyphae; thecium 120–140 μ tall; paraphyses slender, filiform, cells almost isodiametric above, disintegrating into the brown epithecial gel; asci cylindrical, 20 μ in diameter, 8-spored; ascospores imbricately monostichous, broadly fusiform, 23–32 x 10–13 μ, with 5 transverse septa.

This species seems to be widespread in the American tropics, from 700 to 2100 m., rarely also below these levels. *L. stipitatum*, with which this species is easily confused, seems confined to the lower levels, reaching up to about 800 m., and has so far only been reported from Guadeloupe and Costa Rica.

Limón: Waldeck, *Dodge & Nevermann 7938;* Marta, 20 m., *Dodge 7427.*

Cartago: Angostura, *Polakowsky 456;* Juan Viñas, 1000 m., *Calvert 68;* Santiago, 1140–1180 m., *Dodge 4555;* R. Birrís, 1220–1340 m., *Dodge & Thomas 4628;* Aguacaliente, 1240–1460 m., *Dodge & Thomas 7082;* Carpintera, 1700 m., *K. Danielson 101a.*

San José: S. Pedro de Montes de Oca, 1200 m., *Thomas 4717;* S. Juan Tibas, 1000–1100 m., *Dodge 4303.*

Guanacaste: H. Santamaría, 640–680 m., *Dodge & Thomas 6797.*

Puntarenas: Boruca, 560 m., *Tonduz 5380.*

Var. DIGITATUM Eschw. ap. Martius, Fl. Brasil. **1**: 238. 1833.

Collema bullatum Raddi, Atti Soc. Ital. **18**: 36, *pl. 4, f. 2.*

Collema bullatum var. *dactylinoideum* Nyl., Flora **41**: 338. 1858; Syn. Meth. Lich. **1**: 129. 1858.

Type: Brasil, between Mandiocca and Morro do Frade, *Raddi,* type of var. *dactylinoideum* not cited, but specimens from Mexico, *Fr. Müller,* Colombia, Tolima, *Goudot,* and Bolivia, *Weddell,* are mentioned.

This variety is stated by Eschweiler to have a thicker thallus,

deep green instead of lead color, densely wrinkled instead of granular; podetia cylindrical instead of ventricose, 6–12 mm. instead of 2–6 mm. tall, about 6 mm. in diameter.

From the description this variety does not seem distinct from the species and I have seen no material referable here from Costa Rica. It is possible that Eschweiler was attempting to separate this species from *L. phyllocarpum* which he called *Collema* (*Leptogium*) *bullatum* var. *sertatum*.

LEPTOGIUM STIPITATUM Vainio, Hedwigia **38:** (255). 1899.

Type: Guadeloupe Island, near Gourbeyre, *P. Duss 434*.

Thallus plumbeous, soon discolored buffy citrine to Saccardo's olive, irregularly dichotomously branched, ultimate lobes 2–7 mm. broad, then crowded and confluent, rounded, acutely and irregularly wrinkled above and below; about 200 μ thick between the ridges, with *Nostoc* filaments densely coiled and tangled near the surface, mostly parallel to the surface within, much more abundant than in *L. vesiculosum*, with cortex of isodiametric cells on each surface. Apothecia on short thalline pustules, up to 2.5 mm. in diameter, margin thick with periclinal folds (never radial as in *L. vesiculosum*), disc chestnut; amphithecium about 200 μ thick, of the same texture as the thallus; parathecium 80 μ thick, disappearing above, of large-celled pseudoparenchyma; hypothecium 80 μ thick, of more or less parallel hyphae continued upward as a false parathecium; thecium about 140 μ tall; paraphyses filiform, about 1.5 μ in diameter, septate, with capitate tips in the brown epithecial gel; asci cylindrical, 16–20 μ in diameter; ascospores distichous, broad-fusiform with acute ends, 24–32 x 10–12 μ.

San José: cañon of R. Virilla, below El Brazil and Sta. Ana, *Dodge 8019*.
Puntarenas: Peninsula of Puntarenas, 3–5 m., *Dodge 8021*.

LEPTOGIUM PHYLLOCARPUM (Pers. ap. Gaudich.) Mont., Ann. Sci. Nat. Bot. III, **10:** 134. 1848.

Collema phyllocarpum Pers. ap. Gaudich. in Freycinet, Voy. Uranie, Bot. 204. 1826.

Collema bullatum var. *sertatum* Eschw. ap. Martius, Fl. Brasil. **1:** 239. 1833.

Type: Brasil, Rio de Janeiro, *Gaudichaud*.

Thallus plumbeous but frequently becoming black, especially

in specimens which have been dried slowly or repeatedly wetted and dried after collection, lobes broad, surface deeply and acutely wrinkled, the wrinkles predominantly longitudinal or radial, the secondary wrinkles very irregular; more than 200 μ thick between the wrinkles, corticate on both surfaces by a single layer of isodiametric cells, the filaments of *Nostoc* loosely tangled, more or less parallel to the surface and much more coiled and densely tangled just under the upper cortex. Apothecia about 2 mm. in diameter, margin with crowded periclinal wrinkles, disc rufous, immersed in hollow protuberances as in *L. vesiculosum;* amphithecium appearing lobed in section up to 500 μ thick, homogeneous with the thallus but algae a little denser; parathecium about 100 μ thick below, thinning out to about 20 μ above at the margin, of large-celled pseudoparenchyma; hypothecium about 30 μ thick, of slender densely woven hyphae continuing up to the margin at about the same width; thecium about 140 μ tall; paraphyses filiform, not dilated above, epithecial gel brown; asci cylindric, about 20 μ in diameter, 8-spored; ascospores broad-fusiform with acute ends, monostichous, with 5 transverse septa, 22–30(–35) x 10–12(–14) μ.

Apparently this species is very widespread and common in the American tropics from low elevations in the southern United States to 2500 m. in Perú. In Costa Rica it ranges from 500 m. to about 1900 m., being characteristic in the moister fog-bathed areas of the temperate region.

Cartago: Santiago, 1140–1180 m., *Dodge 8000;* R. Birrís, 1220–1340 m., *Dodge & Thomas 7994.*

San José: Cañon of R. Virilla below El Brazil, *Dodge 7782;* Zapote, 1200 m., *Standley 40273;* La Palma, 1500–1700 m., *Maxon & Harvey 7891;* Quebradillas, near Sta. María de Dota, 1800 m., *Standley 42977;* Sta. María de Dota, 1500–1800 m., *Standley 42424;* S. Marcos de Dota, 1200 m., *Tonduz 5374;* R. Naranjo, *Tonduz 5375;* S. Gabriel, *Tristan 5229;* S. José, *Polakowsky 113.*

Alajuela: R. Ciruela, 920–980 m., *Dodge & J. Valerio 4858;* Santiago de S. Ramón, 1000 m., *Brenes;* Viento Fresco, 1600–1900 m., *Standley & Torres 47771.*

? Puntarenas: Corozal, 5–50 m., *Dodge 8025.*

Var. MACROCARPUM Nyl., Syn. Meth. Lich. **1**: 130. 1858.

Type: not cited but specimens from Aequatorial America, *Humboldt,* Chile, Perú, and Venezuela, *Lind. 1092,* are mentioned.

Similar to the species but apothecia large, 5–9 mm., margins lobulate, foliose. American material referred to var. *daedaleum*

Nyl. probably belongs here. Distribution in Costa Rica essentially similar to that of the species but not yet collected in the higher elevations.

Cartago: Santiago, 1140–1180 m., *Dodge 8001;* R. Birrís, 1220–1340 m., *Dodge & Thomas 4632;* Carpintera, 1700 m., *K. Danielson 101b.*

San José: R. Naranjo, *Tonduz 5377.*

Alajuela: La Palma de San Ramón, 1250 m., *Brenes 91.*

Guanacaste: H. Santamaría, 640–780 m., *Dodge & Thomas 6776, 8002;* Tilarán, 500–690 m., *Standley & J. Valerio 44803, Dodge & Thomas 6562.*

Puntarenas: Corozal, 5–50 m., *Dodge 7529.*

Var. CAMPESTRE Malme, Ark. f. Bot. **19**[8]: 13. 1924.

Type: Brasil, Bahia, R. Vermelho, *Malme 29, 30;* Matto Grosso, Cuyaba, *Malme 2715c,* etc.; Serra da Chapada, pr. Bocca da Serra, *Malme 2245B.*

Differs from the typical form by narrower crowded lobes, margins crisped; apothecia 1–2 mm. in diameter, margin rugulose, thin; amphithecium corticate with a single layer of cells; parathecium 60–70 μ thick, reaching the margin above; thecium 100–125 μ tall, asci up to 20 μ thick, 8-spored; ascospores irregularly distichous, broad-fusiform, acute ends, 22–27 x 8–10 μ, usually with 5 transverse septa.

San José: Turrúcares, 540–600 m., *Dodge & Thomas 7481.*

Alajuela: R. Ciruela, 920–980 m., *Dodge & J. Valerio 7921;* Santiago de S. Ramón, 1000 m., *Brenes.*

LEPTOGIUM CORALLOIDEUM Vainio, Ann. Acad. Sci. Fenn. A **6**[7]: 110. 1915.

Leptogium diaphanum f. *coralloideum* Mey. & Fw., Nova Acta Acad. Leopold. Carolin. **19**: Suppl. 226. 1843.

Leptogium phyllocarpum var. *isidiosum* Nyl., Syn. Meth. Lich. **1**: 130. 1858.

Leptogium phyllocarpum var. *coralloideum* Hue, Nouv. Arch. Museum Paris III, **10**: 228. 1898.

The type of var. *isidiosum* is from Mexico, Orizaba, *Fr. Müller.*

Thallus as in *L. phyllocarpum* but densely isidiose, especially along the wrinkles, somewhat thicker, semi-pellucid. Apothecia large when present, very rare, margin densely coralloid-isidiose, disc rufous; amphithecium highly developed, homogeneous with the thallus; parathecium 140 μ thick, disappearing above, of large thin-walled pseudoparenchyma; hypothecium 60 μ thick, reaching the margin above, of densely woven slender hyphae;

thecium 200 μ tall; paraphyses filiform with clavate apices; asci cylindrical; ascospores 8 per ascus, imbricately monostichous, ellipsoid to fusiform, apices acute, 30–40(–45) x 12–17 μ, with 7 transverse septa.

This species is perhaps only a large isidiose variety of *L. phyllocarpum*, essentially similar in morphology but larger in most dimensions. It occupies the same general region as *L. phyllocarpum*. It is usually sterile and hence not easily distinguished from sterile *L. marginellum* var. *isidiosellum*. Material has been seen from Mexico, Perú, and Brasil.

Limón: Hamburg, 20–30 m., *Dodge 7420.*

Cartago: Pejivalle, 650–900 m., *Standley & J. Valerio, Dodge & Thomas 4337;* Cañon of R. Reventazón below Santiago, 920–1000 m., *Dodge & Thomas 8013, Dodge 8004;* R. Birrís, 1220–1340 m., *Dodge & Thomas 7954;* C. Carpintera, 1320–1700 m., *Dodge 8758, Dodge & Thomas 4762, K. Danielson 98;* Aguacaliente, 1240–1460 m., *Dodge & Thomas 8003;* R. Reventado, 1460–1650 m., *Standley & J. Valerio 49614.*

San José: R. Virilla below El Brazil, *Dodge 6496;* Zurquí, 1600–2500 m., *Standley & J. Valerio 48255, 48266, 48146, Dodge, J. Valerio & Thomas 6042.*

Alajuela: near Fraijanes, 1500–1700 m., *Standley & Torres 47425, 47462.*

Guanacaste: H. Santamaría, 640–680 m., *Dodge & Thomas 8005;* Tilarán, 500–650 m., *Standley & J. Valerio 44367, 44403.*

LEPTOGIUM MARGINELLUM (Sw.) S. F. Gray, Nat. Arrang. Brit. Pl. 1: 401. 1821.

Lichen marginellus Sw., Nov. Gen. Sp. Pl. Prodr. 147. 1788.

Collema vesicatum Taylor, London Jour. Bot. 6: 196. 1847.

Leptogium corrugatulum Nyl., Syn. Meth. Lich. 1: 132. 1858.

Type: Jamaica, Blue Ridge Mt., *Swartz.* The type of *C. vesicatum* Taylor is from St. Vincents, West Indies. The type of *Leptogium corrugatulum* is from Mexico, Jalapa, *Galeotti 9630.*

Thallus plumbeous, round-lobed, margin smooth or occasionally microphylline, pellucid, surface of undulating longitudinal wrinkles less acute than in *L. phyllocarpum*, of variable thickness, about 160 μ thick, corticate on both surfaces with small isodiametric cells. Apothecia minute, up to 0.3 mm. in diameter, margin densely isidiose, disc rufous; amphithecium thick, with pseudoparenchymatous cortex below, a single layer of cells above, as are also the isidia; parathecium 150 μ thick, pseudoparenchymatous; thecium 150–180 μ tall, epithecium tawny; asci 8-spored; spores imbricately monostichous, ellipsoid-fusiform, ends acute, 25–35 (–40) x 10–13 μ, with 5 transverse septa.

This species rarely matures spores although rudimentary apothecia are nearly always abundant. The microscopic details of the apothecium are largely taken from Malme as I have seen no mature asci in my Costa Rican material. There is considerable variation in the amount of isidia, as they easily break off leaving minute foveoles on the margin. The species is quite distinct and is widespread in the American tropics. I have seen material from Florida, Alabama, Bermuda, various islands of the West Indies, Mexico, Brasil, and the Galápagos Islands. It seems to range from sea level to 1500 m.

From the nature of the isidia it seems likely that var. *isidiosellum* Riddle, Brooklyn Bot. Gard. Mem. **1**: 115. 1918, should be referred to *L. coralloideum*, although sterile isidiose states are very difficult to place.

Limón: Marta, 20 m., *Dodge & Nevermann 7986;* Carmen, *Dodge 7422;* Waldeck, *Dodge & Nevermann 7421.*

Cartago: Pejivalle, 600–900 m., *Dodge & Thomas 8014, Standley & J. Valerio 46769;* R. Birrís, 920–1100 m., *Dodge 7988;* Santiago, 1140–1180 m., *Dodge 7987;* Aguacaliente, 1240–1460 m., *Dodge & Thomas 7937;* Carpintera, 1500 m., *Dodge 3948.*

Alajuela: Santiago de S. Ramón, 1000 m., *Brenes 242;* S. Pedro de S. Ramón, 700 m., *Brenes.*

Guanacaste: H. Santamaría, 640–720 m., *Dodge & Thomas 6867, 7989;* H. Granadilla, above R. S. José, 480 m., *Dodge & Thomas 7936;* H. Q. Azul, on lower slope of V. Tenorio, 400–600 m., *Dodge & Thomas 6653;* Tilarán, 500–690 m., *Dodge & Thomas 6561.*

Puntarenas: R. Terrones, 30 m., *Dodge & Marks 7980;* Corozal, 5–50 m., *Dodge 7533.*

LEPTOGIUM DENTICULATUM Nyl., Ann. Sci. Nat. Bot. V, **7**: 302. 1867.

L. tremelloides v. *leptophyllinum* Mey. & Fw., Nova Acta Acad. Leopold. Carolin. **19**: Suppl. 228. 1843.

L. tremelloides v. *azureum* f. *isidiosum* Müll. Arg., Bull. Soc. R. Bot. Belg. **29**: 49. 1891, non Flora **65**: 292. 1882.

L. leptophyllinum Zahlbr., Cat. Lich. Univ. **3**: 136. 1924.

Type: Colombia, San Jil, 1300 m., *Lindig.*

Thallus mineral gray or darker, large, broadly lobed, lobes round, edges smooth, somewhat crisped, surface slightly wrinkled, 100–120 μ thick, microphylline, isidiose, corticate on both sides with a single layer of cells, the short filaments of *Nostoc* with cells 4–5 μ in diameter mostly assembled just under the cortex,

leaving the central portion relatively free of algae. Apothecia relatively rare but when present usually numerous, scattered over the upper surface, up to 2 mm. in diameter, margin granular, thick, becoming isidiose in age, buff, disc rufous; amphithecium 220 μ thick, with a layer of pseudoparenchymatous cortex below about 160 μ thick, not thinning out much above and merging with the parathecium, algal layer 60 μ thick below, thinning out to 30 μ above; parathecium 100 μ thick below, thinning out to about 40 μ above, of large thin-walled hyphae at times suggesting pseudoparenchyma; hypothecium 20 μ thick, of more slender deeply staining hyphae; thecium 160 μ tall; paraphyses slender, ending abruptly in the epithecial gel; asci 10–12 μ in diameter, cylindrical, 8-spored; ascospores imbricately monostichous, fusiform with acute ends and about 5 transverse septa, about 24 x 10 μ.

This species seems widespread in the American tropics, although it is so rarely fertile that it is difficult to be sure of the determination of much of the sterile material, especially to separate it from *L. cyanescens* var. *austro-americanum* Malme. As here described, this species is close to, although not identical with, Malme's variety, while the *L. denticulatum* Malme seems to be an isidiose state of or closely related to *L. Tuckermani*. Only a careful comparison of all the fertile specimens from tropical America in the various herbaria of Europe can settle these problems.

Limón: Marta, 20 m., *Dodge & Nevermann 7428;* Hamburg, *Standley & J. Valerio 48750;* Castilla, 20 m., *Dodge & Nevermann 7996;* Waldeck, *Dodge & Nevermann 7997, Dodge 8012.*

Cartago: R. Reventazón below Santiago, 740–750 m., *Dodge 4626,* 920–1000 m., *Dodge & Thomas 7993;* Santiago, 1100–1140 m., *Dodge 7939;* R. Birrís, 1220–1340 m., *Dodge & Thomas 7992;* Estrella, 1600 m., *K. Danielson 162;* near Cartago, 1500 m., *K. Danielson 22;* C. Carpintera, 1500 m., *Dodge 3973.*

San José: San José, *Tonduz 5247.*

Heredia: C. Zurquí, 1600–1700 m., *Dodge, J. Valerio & Thomas 8048.*

Alajuela: C. Mondongo de S. Ramón, 750–800 m., *Brenes 275;* Alto de la Palma de S. Ramón, 1250 m., *Brenes 377.*

Guanacaste: H. Santamaría, 680–780 m., *Dodge & Thomas 6870, 7014;* H. Granadilla above R. Las Cañas, 500–600 m., *Thomas 6623.*

Puntarenas: Osa, Corozal, 5–50 m., *Dodge 7998.*

LEPTOGIUM AZUREUM (Sw.) Mont. ap. Webb, Hist. Nat. Îles Canaries 3[1]: 129. 1840.

Lichen azureum Sw. ap. Ach., Lichenog. Suec. Prodr. 137. 1798.

Type: mountains of Jamaica, *O. Swartz.*

Thallus pellucid, greenish-glaucous-blue to puritan gray, broadly sinuate-rounded lobes, smooth; margins smooth, 80–100 μ thick, of loosely coiled and tangled filaments of *Nostoc* with ellipsoidal cells 2.5 x 4 μ. Apothecia scattered, 1–2 mm. in diameter, margin smooth, light buff, disc rufous, sessile or on a short stalk; amphithecium 320 μ thick, below with a pseudoparenchymatous cortex about 100 μ thick, above with a single layer of isodiametric cells in the cortex; parathecium filamentous, 40 μ thick, poorly developed, of slender hyphae not reaching the margin; hypothecium 20 μ thick, deeply staining; thecium 120–150 μ tall; paraphyses filiform, 1.5–2 μ thick, thickened at the tips to about 4 μ; asci clavate-cylindrical, about 18 μ in diameter, 8-spored; ascospores imbricately monostichous or somewhat distichous, fusiform ends acute, with 3 or 5 transverse septa, 22–28 x 8–10 μ.

Only a careful study of all the types involved can settle nomenclature of the group of species centering around *L. tremelloides*. This species was originally collected in South Africa by Thunberg, and very briefly described by Linné fil. The early lichenologists of the west coast of Europe identified their material as this species and later reduced Swartz' Jamaican *L. azureum* to synonymy. There is apparently a whole group of species having approximately the same macroscopic appearance but wholly different microscopic structure which are at present referred to this species by various workers. As we have here defined it (agreeing closely with *L. tremelloides* Malme), it is a species of the lowlands coming up the river valleys under favorable conditions to about 1000 m. in Costa Rica. It seems widely distributed at lower elevations in tropical America.

Limón: Marta, 20 m., *Dodge & Nevermann 7414, 7426;* Hamburg, 20–30 m., *Dodge & Nevermann 7417;* Castilla, 20 m., *Dodge 7209, 7419, 7424;* Carmen, *Dodge 7415;* Indiana I. near Siquirres, 60–70 m., *Dodge & Thomas 5572;* along R. Siquirres, 70–350 m., *Dodge, Catt & Thomas 8006, 8007, 8008;* Livingston, 80–100 m., *Dodge, Catt & Thomas 5573;* Waldeck, *Dodge & Nevermann 7412, 7413, 7416;* Guápiles, 300–500 m., *Standley 37137.*

Cartago: Turrialba, *Ørsted;* R. Pejivalle, 650–800 m., *Dodge & Thomas 4414;* R. Reventazón, 920–1000 m., *Dodge & Thomas 4604.*

Guanacaste: H. Santamaría, 760–900 m., *Dodge & Thomas 6876;* Tilarán, 500–690 m., *Dodge & Thomas 8009.*
Puntarenas: Osa, C. Guaca, 85 m., *Dodge 7505;* Corozal, 5–50 m., *Dodge 7530.*
Isla Coco: *Snodgrass & Heller.*

LEPTOGIUM **Standleyi** Dodge, sp. nov.

Leptogium tremelloides Auct., non Linné fil.

Type: Costa Rica, La Hondura, *Standley 37870.*

Thallus plumbeus, crassus, laevis vel minute rugulosus, lobis rotundatis, latis, marginibus integris, 150–170 μ crassitudine, filamentis nostocaceis sub cortice dense implicatis, centro laxe aut sparse implicatis, corticatum e serie simplice cellularum. Apothecia ad 3 mm. latitudine, plana, margine integro, tenui, laevi, fulva, disco rufo; amphithecium inferne 160 μ, ad 100 μ crassitudine superne in margine attenuatum, cortice pseudoparenchymatico, 90 μ crassitudine inferne ad una serie cellularum superne attenuatum, filamentis nostocaceis dense contextum; parathecium 60 μ crassitudine, hyphis tenuibus dense contextum; hypothecium flavum, 20–30 μ crassitudine, hyphis tenuibus dense contextum; thecium ad 180 μ altitudine; paraphyses filiformes, tenues, apice non incrassata; asci cylindrici, 20 μ diametro metientes; ascosporae octonae, imbricatim monostichae vel distichae, fusiformes, 24–28 x 12 μ.

Thallus mineral gray or darker, thick, smooth or minutely and shallowly wrinkled, lobes broad, rounded, margins smooth, 150–170 μ thick, filaments of *Nostoc* densely tangled next the cortex, loosely tangled and scattered in the center of the thallus, cortex of a single series of cells on both surfaces. Apothecia 3 mm. broad, plane, margin smooth, thin, tawny, disc rufous; amphithecium 160 μ thick below, thinning to 100 μ above at the margin, corticate below, 90 μ thick, pseudoparenchymatous, above becoming a single layer of cells, filaments of *Nostoc* densely tangled; parathecium 60 μ thick, of slender more or less parallel hyphae; hypothecium yellowish, 20–30 μ thick, of slender densely woven hyphae; thecium 180 μ tall; paraphyses filiform, slender, apices not thickened; asci cylindrical, 8-spored, 20 μ in diameter; ascospores imbricately monostichous or distichous, fusiform, 24–28 x 12 μ.

This species is essentially close to *L. azureum* in its microscopic characters but differs in its much larger dimensions, its thicker,

opaque thallus, and larger apothecia. Superficially it somewhat resembles *L. pulchellum* but is much less coarsely wrinkled and differs in microscopic details. In Costa Rica it seems to be confined to the higher elevations reaching 1700 m. It is probable that material determined as *L. pulchellum* from Costa Rica by Müller Argau belongs here, although I did not have time to section it. It certainly is not typical *L. pulchellum*.

Cartago: Turrialba, *Ørsted;* Orosí, 1000–1100 m., *Standley 39791.*

San José: San José, 1130 m., *Standley 41233;* La Hondura, 1300–1700 m., *Standley 37870,* TYPE.

Alajuela: Poás, *Tonduz 5301.*

LEPTOGIUM **Tuckermani** Dodge, sp. nov.

Leptogium tremelloides var. *minor* Tuck. in herb.

? *Leptogium moluccanum* Vainio, Étude Lich. Brésil 1: 223–224. 1890, non *Collema moluccanum* Pers. ap. Gaudich. in Freycinet, Voy. Uranie, Bot. 203. 1826.

Type: Cuba, Monte Verde, *C. Wright 56.*

Thallus plumbeus vel obscurior, lobis confertis, rotundatis, superne inferneque sat laevigatus, tomento isidiisque destitutus, tenuissimus, 35–60 μ crassitudine, strato nostocaceo 25 μ, corticibus pseudoparenchymaticis cellulis magnis. Apothecium minutum, 0.5–0.9 mm. diametro metiens, peltatum, basi constrictum, margine integro, pallescente, disco castaneo; amphithecium corticatum cellulis pseudoparenchymaticis, 60 μ crassitudine inferne, attenuatum ad 12 μ superne, strato nostocaceo 40 μ crassitudine; parathecium pseudoparenchymaticum tenue, 10–12 μ crassitudine, cellulis parvis; hypothecium 20 μ crassitudine, hyphis tenuibus dense contextum; thecium 90–120 μ altitudine; paraphyses filiformes, 2 μ diametro, apicibus incrassatis; epithecium brunneum; asci cylindrici, 12 μ diametro metientes; ascosporae octonae, imbricatim monostichae, apicibus acutis, murales, septis transversalibus 3–5, cellulis haud numerosis, 18–22 x 10–12 μ.

Thallus mineral gray or darker, lobes crowded, more or less elevated and crisped, rounded, smooth on both sides, 35–60 μ thick, algal layer of *Nostoc* about 25 μ, with pseudoparenchymatous cortex above and below of large cells. Apothecia minute, 0.5–0.9 mm. in diameter, constricted at the base, margin whole, smooth, pale, disc chestnut; amphithecium with a thick pseudo-

parenchymatous cortex below, about 60 μ, thinning to two rows of cells about 10–12 μ thick above; parathecium thin, pseudoparenchymatous, 10–12 μ thick, of small cells, inconspicuous and often reported absent; hypothecium 20 μ thick, of slender densely woven hyphae; thecium 90–120 μ tall; paraphyses filiform, 2 μ in diameter, with clavate apices; epithecium brown; asci cylindric, 12 μ in diameter; ascospores 8 per ascus, imbricately monostichous, ends acute, muriform, with 3–5 transverse septa, cells not numerous, 18–22 x 10–12 μ.

This species has long been confused with *L. moluccanum*, *L. Mariannum*, and *L. diaphanum*. It differs from the two former in habit and color and from the latter by its structure. It is apparently widespread in the American tropics, being reported from Paraguay, and from Brasil in the states of Rio Grande do Sul and Matto Grosso by Malme, and in Minas Geraes by Vainio.

Limón: Hamburg, *Dodge 7425*.

Cartago: Pejivalle, 680 m., *Dodge & Thomas 4556;* Santiago, 1140–1180 m., *Dodge 4555, 8044;* Las Concavas, 1350–1450 m., *Dodge 6842*.

San José: hills above Sta. Ana, *Dodge 7781*.

Guanacaste: H. Santamaría, 680–780 m., *Dodge & Thomas 8043;* H. Granadilla, 480 m., *Dodge & Thomas 8045*.

LEPTOGIUM SIMPLICIUS Vainio, Ann. Acad. Sci. Fenn. **A6**[7]: 109. 1915.

Type: S. Domingo, La Cumbra, *C. Raunkiaer*.

Thallus between light drab and light grayish olive, with irregularly incised lobes 5–7 mm. broad, not isidiose, with slightly undulate elevated acute wrinkles on both surfaces, corticate with a single layer of isodiametric cells. Apothecia broadly adnate, constricted at the base, sessile, 1.5–2.2 mm. in diameter, disc plane, rufous, margin thin, smooth, lighter than the thallus; amphithecium 140–150 μ thick, corticate with a single series of cells above, pseudoparenchymatous below, 90–100 μ thick; parathecium 120 μ thick below, thinning out to 20 μ thick at the margin above, pseudoparenchymatous; hypothecium filamentous, 30 μ thick; thecium 150–170 μ thick; paraphyses filiform, tips somewhat thickened in the brownish epithecial gel; asci cylindric, 14–16 μ in diameter, 8-spored; ascospores imbricately monostichous, fusiform, thin-walled, with 5 transverse septa, 22–30 x 11–13 μ.

This species previously reported only from the type locality has yet been found only at low elevations on the Pacific slope.

San José: Turrúcares, 540–600 m., *Dodge & Thomas 8047.*
Guanacaste: Liberia, 100 m., *Dodge, Alfaro & Thomas 6586, Dodge & Thomas 8028,* Tilarán, 500–690 m., *Dodge & Thomas 8046.*

Var. **pichneoides** Dodge, var. nov.

Type: Costa Rica, Guanacaste, H. Santamaría, *Dodge & Thomas 8029.*

Thallus isidiosus, marginibus microphyllinis aut isidiosis. Apothecium margine tenuiore, laevi, thecium 130–150 μ altitudine.

Thallus isidiose, margins varying from dentate to short isidiose or slightly microphylline. Apothecia with thinner, smooth margins, thecium 130–150 μ tall.

Only the type has been found fertile and here there are very few apothecia. The other collections cited probably belong here, but it is always difficult to determine sterile isidiose specimens in this genus.

Alajuela: R. Ciruela, 920–980 m., *Dodge & J. Valerio 4894.*
Guanacaste: H. Santamaría, 640–680 m., *Dodge & Thomas 8029.*

Besides the species cited above, a single small fragment was found which seems to belong in *Leptogiopsis*, but more material is necessary for a satisfactory disposition.

PANNARIACEAE

Thallus squamose or foliose, not gelified; hypothallus and rhizinae usually highly developed, heteromerous, cortex of erect, irregular or periclinal hyphae, usually more or less pseudoparenchymatic; medulla well developed, with *Nostoc, Scytonema,* or *Dactylococcus;* lower cortex of thick-walled periclinal hyphae often thin or wholly absent. Apothecia marginal or scattered over the upper surface, biatorine or lecanorine; paraphyses unbranched; asci 8-spored; spores hyaline, unicellular, rarely 2–4-celled; spermatia short, straight.

This family seems to be of southern origin with some genera confined to the southern hemisphere, while a few species come northward until they are circumpolar. In general, the northern species are much smaller and more depauperate than the southern species.

In many ways the family seems very homogeneous, so that

genus distinctions are often rather arbitrary. Hue, in 1912, reduced the whole family to a single genus, but his sections and subsections correspond more or less closely to the genera of earlier workers. Zahlbruckner has recognized twelve genera, although the generic delimitation is not altogether clear. Following this treatment I have tentatively recognized seven genera from Tropical America. *Psoroma*, with *Dactylococcus* algae, is largely confined to the southern hemisphere, and by many authors has been placed in the Lecanoraceae. *Lepidocollema*, with a thin upper cortex and almost homoeomerous, is very close to the Collemaceae and is known from a single collection in Brasil. The other five genera are much better known and widespread in the American tropics.

The morphology of the thallus is not altogether clear and the nomenclature of the parts is rather confusing. In the ideal case, the uppermost layer of the thallus, here called "tomentum," is a mass of loosely woven hyphae formed by proliferation of deeper layers. In *Erioderma* it is soft and spongy, like a loose felt, while in *Malmella* and in some of the isidiose members of *Pannaria* it has the appearance of a fine blotting paper, or in *Coccocarpia* the hyphae are more conspicuously parallel and conglutinate, suggesting a frayed cotton yarn. In some species of each genus the tomentum is partly evanescent, or occasionally it is conglutinated into spines or warts. Below the tomentum is an upper cortex of pseudoparenchyma, usually formed by the partial disintegration of a palisade of large thick-walled hyphae which occasionally penetrate between the algal filaments. In *Coccocarpia* the pseudoparenchyma is thin and scarcely distinguishable from the tomentum or even absent. The algal zone varies from a dense palisade of filaments of *Scytonema* and hyphae in some species, to ellipsoidal colonies of *Nostoc* surrounded by hyphae. The medulla in most genera consists of loosely tangled hyphae, occasionally not even well differentiated from the algal layer, while in *Coccocarpia* it is formed of septate periclinal hyphae, scarcely to be distinguished from the lower cortex except in color and the size of the intercellular spaces. The lower cortex may be completely absent or consist of one or more layers of thick-walled periclinal hyphae which give rise to

tufts of rhizinae. The so-called hypothallus in most species is a thick dense mat of rhizinae, which may be absent in *Erioderma*, or occasionally only slightly developed in certain species of the other genera.

The morphology of the apothecium is equally confusing. In *Pannaria* and *Malmella* an amphithecium is present, usually with a pseudoparenchymatous cortex, and a thin algal layer which may be separated from the parathecium by a thin medulla. The parathecium is pseudoparenchymatous, formed from thick-walled periclinal hyphae in *Lepidocollema*, *Parmeliella*, *Coccocarpia*, *Erioderma*, and the smooth species of *Pannaria*, while in the isidiose species of *Pannaria* and in *Malmella* the parathecium is filamentous, in the former of large thin-walled cells, in the latter of slender hyphae. The hypothecium is differentiated somewhat in the species of *Pannaria* but practically undifferentiated in the other genera. Instead of the above interpretation those with a filamentous parathecium might be conceived as lacking a parathecium and having a somewhat highly developed hypothecium, or perhaps the so-called parathecium is really only a compact medulla of the amphithecium. Further study of the morphology of the developmental stages, also more representatives of some of the genera, are necessary before the correctness of any interpretation can be assured.

KEY TO THE TROPICAL AMERICAN GENERA OF PANNARIACEAE

Thallus with Chlorophyceae; apothecia lecanorine, rhizinae little developed or absent; Perú ..... *Psoroma cinchonarum*

Thallus with Myxophyceae; rhizinae usually well developed.

Upper cortex thin, pseudoparenchymatous, algal layer occupying most of the thallus; apothecia biatorine; Brasil ..... *Lepidocollema carassense*

Upper cortex well developed, pseudoparenchymatous.

Upper cortex of two or more rows of cells; hyphae perpendicular to the surface, medulla loosely woven.

Apothecia lecanorine.

Tomentum absent or very early evanescent, algae definitely *Nostoc*, colonies usually separate; parathecium usually pseudoparenchymatous ..... *Pannaria*

Tomentum thin but persistent, algae *Nostoc*? or perhaps *Scytonema*; parathecium of slender hyphae ..... *Malmella*

Apothecia biatorine, pseudoparenchymatous from periclinal hyphae.

Tomentum absent, algae *Nostoc*; rhizinae well developed .... *Parmeliella*

Tomentum present and highly developed, algae *Scytonema* or *Nostoc*; lower cortex little developed; apothecia large, marginal ..... *Erioderma*

Upper cortex of septate periclinal hyphae which may simulate pseudoparenchyma, tomentum usually absent; apothecia biatorine, medulla of periclinal conglutinate hyphae appearing pseudoparenchymatous, algae *Scytonema*.....*Coccocarpia*

PANNARIA Del. ap. Bory

PANNARIA Del. ap. Bory, Dict. Class. Hist. Nat. **13**: 20. 1828.

Type species: *Pannaria rubiginosa* (Thunberg ap. Ach.) Del. ap. Bory.

Thallus granular, squamose to foliose with a well-developed bluish black or black hypothallus, rarely with dark, more or less tangled rhizinae below, heteromerous, upper surface corticate with large-celled pseudoparenchyma formed from a palisade of hyphae; algae *Nostoc*, medulla single or double, in the former case arachnoid, in the latter the upper portion of more or less parallel, thin-walled hyphae, loosely woven, and below of densely tangled hyphae, without lower cortex. Apothecia at first sunk in the thallus, finally sessile or peltate, superficial; amphithecium pseudoparenchymatous with a few algae in the center; hypothecium hyaline; asci clavate, 8-spored; ascospores hyaline, elongate, ellipsoidal to almost fusiform with a somewhat thickened and finely verrucose wall. Spermagonia in hemispherical warts, spermatiophores septate with short, broad cells; spermatia straight or very slightly curved, elongate, cylindrical.

This genus seems one of the most variable and widespread of the family, occurring from the Arctic to the Antarctic, in Costa Rica occurring from sea level to 1700 m.

In tropical America the genus separates easily into two sections, the isidiose species with a filamentous parathecium, and the smooth species with a pseudoparenchymatous parathecium.

KEY TO THE TROPICAL AMERICAN SPECIES OF PANNARIA

Hypothallus not well developed, pale with pale rhizinae below.
- Thecium 80–100 μ or less tall; parathecium filamentous; hypothecium not differentiated; spores 11–12 x 4–5 μ, more or less constricted at the middle and appearing 2-celled; S. Africa.....*P. rubiginosa*
- Thecium 100–120 μ or more tall; parathecium of septate hyphae; hypothecium pseudoparenchymatous; spores 13–16 x 7–9 μ; Rio Grande do Sul, Brasil [*P. rubiginosa* Malme non aliorum].....*P. Malmei*

Hypothallus developed, sometimes sparingly.
- Hypothallus pale, thallus squamulose, pale olivaceous to rusty yellow, margin cut-crenate, irregularly lobulate; spores 8–9 x 2.5–3 μ; Mexico.....*P. applanata*

Hypothallus black.
Thallus isidioid.
Margins and surface with cylindrical, coralloid branched isidia; spores 14–19 x 7–8.5 μ; Antilles and Brasil.....*P. stylophora*
Margins isidio-lacinulate; spores 8–10 x 5–6 μ, with thick epispore; Minas Geraes, Brasil.....*P. isidioidea*
Margins with verruciform isidia, lobes under 1 mm. broad; apothecia 1–1.5 mm. in diam.; spores 12–14 x 8–10 μ, wall thin and smooth. .....*P. Moseni*
Thallus without isidia or soredia.
Thallus livid fuscescent, radiately broad-lobed; spores 14 x 6 μ; hypothallus less developed, center glebulose-lobed; São Paulo, Brasil. .....*P. imbricatula*
Thallus pallid or ashy or pale glaucous, lobes subcuneate, up to 2 mm. broad; spores 12–15(–18) x 8–9(–10) μ; parathecium and hypothecium pseudoparenchymatous.....*P. Vainii*
Thallus coeruleo-ashy, lobes cuneate obovate; epithecium black aeruginous; spores 12–20 x 7–8 μ, surface subplicate; São Paulo, Brasil.....*P. caeruleo-nigricans*
Thallus livid glaucescent or lurid pallid, laciniae very distinctly radiate-stellate, even subimbricate.....*P. radiata*
Thallus pale fuscescent, lobes 2–2.5 mm. broad and about 6 mm. long; epithecium rufous; spores 12–19 x 8–10 μ; parathecium pseudoparenchymatous; hypothecium of conglutinate vertical hyphae; Brasil.....*P. brasiliensis*

PANNARIA ISIDIOIDEA Vainio, Ann. Acad. Sci. Fenn. A6⁷: 102. 1915.

Pannaria Mariana var. *isidioidea* Vainio, Étude Lich. Brésil 1: 206. 1890, excl. syn.

Type: Brasil, Minas Geraes, Sitio, 1000 m., *Vainio 669, 983* (sterile). The following description is based on fertile specimens from Costa Rica.

Hypothallus of dark green rhizinae extending only slightly beyond the thallus; thallus dark olive buff, margins lighter, surface very minutely tomentose, pinnatifid dissected, lobes somewhat cuneate, about 1 mm. wide, ultimate lobules rounded, 0.25–0.5 mm. broad, toward center becoming microphylline and subisidiose but isidia somewhat flattened, rarely cylindric, 150–160 μ thick, upper 40 μ of pseudoparenchymatous cortex, algal layer of *Nostoc* about 40 μ thick, and medulla 40 μ thick, of loosely woven hyphae with occasional small chains of *Nostoc;* rhizinae thick-walled, black, 6–8 μ in diameter. Apothecia crowded, peltate, constricted at the base, about 1 mm. in diameter, margin prominent, crenulate, almost lobulate, disc

chestnut; amphithecium 200 μ thick, of the same texture as the cortex and algal layer of the thallus; parathecium 60 μ thick, filamentous, of large thin-walled periclinal hyphae, ending above in a false pseudoparenchyma; hypothecium about 20 μ thick, of similar structure but more deeply staining; thecium 80–100 μ tall; paraphyses slender, filiform, somewhat clavate above in the brownish epithecial gel; asci clavate, 12 μ in diameter; ascospores ellipsoidal with a thick epispore, 8–10 x 5–6 μ (perhaps still immature).

This species is somewhat variable in appearance and perhaps should be separated into varieties and forms but I have not seen sufficient material to do so properly. Sterile material has been referred here solely on the structure of the thallus. *Dodge & Thomas 6558* from Tilarán has a white reticulate cortex.

Cartago: R. Birrís, 1220–1340 m., *Dodge & Thomas 4559.*
Heredia: C. Central de Zurquí, 1600–1700 m., *Dodge, J. Valerio & Thomas 6048.*
Alajuela: Piedades de S. Ramón, 900 m., *Brenes 875.*
Guanacaste: H. Santamaría, 640–680 m., *Dodge & Thomas 6823, 6911, 6988;* Tilarán, 500–690 m., *Dodge & Thomas 8059, 6558.*

Var. **pulvinata** Dodge, var. nov.

Type: Costa Rica, Alajuela, C. de Pata de Gallo a S. Rafael de S. Ramón, 1200–1250 m., *Brenes.*

Thallus ut in *P. isidioidea* sed isidiosissimus, marginibus adscendentibus, cinerascentibus; isidia coralloidea, elongata, pulvinum formantia.

Thallus as in *P. isidioidea* but densely isidiose with ascending, cinerascent margins; the isidia coralloid, repeatedly branched, crowded, forming a cushion up to 2 cm. in diameter and 3–4 mm. thick.

This variety might easily be mistaken for a sterile pulvinate *Stereocaulon* or a *Siphula*, were it not for the occasional lobe at the margin which shows its relationship.

Alajuela: C. de Pata de Gallo a S. Rafael de S. Ramón, 1200–1250 m., *Brenes.*

PANNARIA **Moseni** Dodge, sp. nov.

Pannaria rubiginosa Malme, Ark. f. Bot. **20**[3]: 7–8. 1924, pro parte.

Type: Brasil, São Paulo, Sororocaba near Santos, *Mosen 3284.*

Thallus albidus, subpinnatifidus, lobis 3–5 mm. longitudine, 0.8 mm. latitudine, apicibus rotundatis, marginibus loborum

verrucosis, vel etiam in centro thalli isidiosus, ad 140 μ crassitudine, cortex superior 30–35 μ crassitudine, pseudoparenchymaticus ex hyphis perpendicularibus formatus, zona nostocacea coloniis subsphericis 35 μ diametro metientibus, medulla ad 60 μ crassitudine hyphis laxe implexis, rhizinae brunneo-nigricantes, hyphis nigris pachydermaticis 3–4 μ diametro, conglutinatis. Apothecia 1–1.5 mm. diametro, marginibus tenuibus, crenato-lobatis et subisidiosis; amphithecium inferne 120 μ crassitudine superne ad 60 μ attenuatum, cortice 40 μ crassitudine, pseudoparenchymatico; parathecium bene evolutum, inferne 40 μ superne ad 60 μ crassitudine, hyphis ad 4 μ diametro leptodermaticis contextum; hypothecium 25 μ crassitudine, hyphis periclinalibus dense compactum; thecium ad 100 μ altitudine; paraphyses filiformes 1–2 μ diametro apicibus clavatis; asci clavati, 25 μ diametro; ascosporae octonae, distichae, 12–14 x 8–10 μ, episporio tenuiusculo.

Thallus pearl gray, subpinnatifid, lobes 3–5 x 0.8 mm., with rounded tips, margins of lobes verrucose or even occasionally cylindric, isidiose toward the center, about 140 μ thick, upper cortex 30–35 μ, pseudoparenchymatous from the breaking up of a palisade layer, algal zone of subspherical colonies of *Nostoc* about 35 μ in diameter, medulla about 60 μ thick, of loosely woven hyphae; rhizinae of brownish black thick-walled conglutinate hyphae 3–4 μ in diameter. Apothecia 1–1.5 mm. in diameter, margins thin, crenate-lobulate or even somewhat isidiose; amphithecium about 120 μ thick below, thinning to about 60 μ above with a cortex about 40 μ thick of pseudoparenchyma; parathecium well developed, about 40 μ below, spreading to 60 μ above, of interwoven thin-walled hyphae about 4 μ in diameter; hypothecium about 25 μ thick of dense periclinal hyphae, staining very deeply; thecium about 100 μ tall; paraphyses filiform, 1–2 μ in diameter, with clavate tips; asci clavate, 25 μ in diameter, 8-spored; ascospores distichous, 12–14 x 8–10 μ, with a somewhat thinner epispore.

It is with some hesitation that I have described this species as new. The thallus is very suggestive of the sterile *P. stylophora*, although it has a somewhat different color and lobing and rather larger dimensions of the parts. It does not seem to be closely

related to *P. rubiginosa*, originally described from Thunberg's South African collections, and is certainly not closely related to the material referred to that species in the North Temperate zone. So far I have only seen this species from the Atlantic Coastal Plain of Costa Rica.

Limón: Hamburg, *Standley & J. Valerio 48702, 48717;* R. Siquirres, 70–200 m., *Dodge, Catt & Thomas 8037.*

PANNARIA STYLOPHORA Vainio, Ann. Acad. Sci. Fenn. **A6**[7]: 102. 1915.

Type: Antilles, Guadeloupe, Sofaga, *P. Duss 1387* (sterile).

Hypothallus black, extending slightly beyond the thallus; thallus with pale olive gray margins and olive gray center, lobes long, slender, oblong, 0.5–0.8 mm. wide, and primary lobes about 15 mm. long, irregularly more or less pinnatifid, smooth above around the margin, the center with scattered to crowded cylindrical isidia which are rarely dichotomously branched, and concolorous, about 125 μ thick, upper cortex 20–25 μ, pseudoparenchymatous from the development of a palisade layer; algal layer about 40 μ thick, consisting of ellipsoidal colonies of *Nostoc* about 30 x 40 μ; medulla about 60 μ thick, loosely woven; lower cortex absent; rhizinae of black thick-walled hyphae about 3–4 μ in diameter.

Limón: R. Siquirres, 70–200 m., *Dodge, Catt & Thomas 8036.*

PANNARIA **Vainii** Dodge, sp. nov.

Pannaria rubiginosa Vainio, Étude Lich. Brésil **1**: 204–205. 1890; Malme, Ark. f. Bot. **20**[3]: 7–8. 1924, quoad spec. *Dusenianum.*

Type: Brasil, Minas Geraes, Sitio, 1000 m., *Vainio 606.*

Hypothallus black, extending 1–2 mm. beyond thallus; thallus between olive buff and smoke gray, irregularly and repeatedly laciniate, lobes 1–2 mm. broad, ultimate lobes short, broader ones up to 4 mm. long, smooth above, not isidiose, about 160–170 μ thick, upper cortex 20–30 μ thick of 2–3 layers of large isodiametric cells; algal layer of spherical to ellipsoidal colonies of *Nostoc* about 60–65 μ in diameter, algae rather large and somewhat angular by mutual pressure, medulla about 80 μ thick of large, thin-walled hyphae, loosely interwoven; rhizinae of black, thick-walled, conglutinate hyphae. Apothecia peltate, 0.8–2

mm. in diameter, margin thick, crenulate, disc rufous; amphithecium 130–440 μ thick, with cortex 20–30 μ thick; parathecium well developed, about 60 μ thick of periclinal large, thin-walled, hyphae simulating pseudoparenchyma; hypothecium about 20 μ thick, of similar structure but of more slender less frequently septate hyphae; thecium 90–100 μ tall; paraphyses 1.5–2.5 μ in diameter, filiform, slightly clavate at the tips in a yellowish brown epithecial gel; asci 14–16 μ in diameter, 8-spored; ascospores distichous, broadly ellipsoidal, 12–18 x 8–10 μ, with thick epispore.

This species is another of the group which in various herbaria is often more or less indiscriminately determined as *P. rubiginosa*, *P. Mariana*, or even *P. nigrocincta*, usually without much critical attention to microscopic details.

Guanacaste: near Tilarán, both Atlantic and Pacific slopes, 500–690 m., *Standley & J. Valerio 44310*, *Dodge & Thomas 6564*, *6565*.

PANNARIA **radiata** (Vainio) Dodge, comb. nov.

Pannaria Mariana f. *radiata* Vainio, Ann. Acad. Sci. Fenn. **A6**[7]: 101–102. 1915, excl. syn.

Type: Antilles, Guadeloupe, *P. Duss*.

Hypothallus brownish black, extending about 1.5 mm. beyond the margin of the thallus, thick; thallus mineral gray with ends of lobes pale smoke gray, dichotomously (rarely trichotomously) divided, laciniae 1 cm. or more long, about 1 mm. broad, tips rounded, reflexed, wholly discrete in outer centimeter, thallus about 90 μ thick, upper cortex of large thin-walled pseudoparenchyma 20–30 μ thick, algal zone of *Nostoc* colonies ellipsoidal, about 40 μ in long diameter, medulla about 20 μ thick of loosely woven hyphae; rhizinae of conglutinate black thick-walled hyphae very closely woven at the lower side of the medulla and performing the function of a lower cortex. Apothecia mostly about 1 mm. in diameter, occasionally up to 2 mm., peltate, margin crenate, of medium prominence, disc rufous; amphithecium 160 μ thick, cortex about 60 μ thick, pseudoparenchymatous, *Nostoc* colonies smaller than in the thallus, separated by strands of pseudoparenchyma; parathecium pseudoparenchymatous, 60–80 μ thick, filamentous, of large very thick-walled hyphae appearing pseudoparenchymatous; hypothecium 15–20 μ thick,

of periclinal, thin-walled, slender hyphae; thecium 120–130 μ tall; paraphyses slender, filiform, septate, without clavate tips; asci 10–12 μ thick, 8-spored; ascospores irregularly distichous, ellipsoidal, 15–16 x 7–8 μ, with a very thick epispore.

This species shows little relationship to *P. Mariana* Fr. of the Old World, a species to which it has been referred. It seems closest to *P. Vainii* but differs in microscopic details and habit.

Limón: Hamburg, *Standley & J. Valerio 48763;* R. Siquirres, 70–200 m., *Dodge, Catt & Thomas 5596.*

MALMELLA Dodge

Malmella Dodge, gen. nov.

Type species: *Erioderma physcioides* Vainio.

Thallus squamosus aut microfoliosus, rhizinis nigris bene evolutus, tomentum superficiei superioris parce evolutum tenue, hyphis implexis sed non conglutinatis ut in *Coccocarpia;* cortex superior pseudoparenchymaticus, algae nostocaceae; medulla hyphis laxe implexis, cortex inferior tenuis hyphis nigris conglutinatis aut nullus. Apothecia lecanorina, sessilia, sparsa; amphithecium adest; parathecium non pseudoparenchymaticum; paraphyses filiformes; asci clavati; ascosporae octonae, ellipsoideae, episporio crasso.

Thallus squamose or small foliose, with a well-developed hypothallus of black rhizinae; tomentum of the upper surface less well developed than in *Erioderma*, of slender hyphae loosely woven, not conglutinate as in *Coccocarpia;* upper cortex pseudoparenchymatous, algae *Nostoc*?, medulla of loosely tangled hyphae, lower cortex of black hyphae as in *Coccocarpia* or wholly absent. Apothecia lecanorine, sessile, scattered over the upper surface, not marginal as in *Erioderma;* amphithecium present; parathecium of slender hyphae, not pseudoparenchymatous; paraphyses filiform; asci clavate, 8-spored; ascospores ellipsoidal, with a thick epispore.

This genus seems intermediate between *Pannaria*, *Erioderma*, and *Coccocarpia*, and collections have been previously referred to each of these genera. In structure of the thallus it seems intermediate between *Pannaria* and *Erioderma*, while in superficial appearance it suggests the more laciniate species of *Coccocarpia.*

KEY TO THE TROPICAL AMERICAN SPECIES OF MALMELLA

Thallus becoming densely isidiose.
  Thallus olivaceous with isidia becoming pruinose and subsorediate, then appearing grayish ..... *M. Randii*
  Thallus white, more laciniate ..... *M. albida*
Thallus not isidiose, medulla not well differentiated from algal zone.
  Thallus white, 120 μ thick, thecium 140–160 μ tall ..... *M. Santamariae*
  Thallus olive buff or darker.
    Thallus olive buff, 120 μ thick, thecium 100–120 μ tall ..... *M. physcioides*
    Thallus dark olive with white pruina about margins, 200 μ thick, thecium 80–90 μ tall ..... *M. caesiocinerea*

MALMELLA **Randii** Dodge, n. sp.

Type: Maine, Mt. Desert Island, *E. L. Rand.*

Hypothallus rhizinis viridi-nigricantibus; thallus olivaceus, lobis subdichotomis aut irregularibus, 1.0–1.3 mm. latis, marginibus crispatis elevatisque, verrucosis et etiam isidioideis, glaber in lobis marginalibus, centro isidiosissimus, apicibus isidiorum pruinosis et subsorediosis; thallus 140–160 μ crassitudine, tomentum 12 μ crassitudine hyphis laxe implexis, tenuibus, subdilabentibus; zona nostocacea [vel scytonematica] 100 μ crassitudine, filamentis dense convolutis, in coloniis ellipsoideis, cellulis sphericis vel angularibus; medulla hyphis laxe implexis 2–3 μ diametro, aut plus minusve hyphis subparallelis ut in *Coccocarpia pellita*, frequenter ad margines; cortex inferior hyphis conglutinatis nigris tenuis.

Hypothallus of well-developed greenish black rhizinae extending about 0.5 mm. beyond the thallus; thallus ecru olive to buffy olive, main lobes subdichotomous or wholly irregular, 1.0–1.3 mm. broad, margin crisped and elevated, becoming verrucose and finally isidioid, surface appearing like surface of blotting paper under microscope, smooth on marginal lobes, becoming densely isidiose toward the center, the tips of the isidia soon becoming pruinose and subsorediate, giving a puritan gray appearance to the plant; thallus about 140–160 μ thick, the tomentum 12 μ thick of loosely appressed, somewhat tangled, slender hyphae which more or less disintegrate; the upper cortex 20 μ thick, of about 2 layers of cells from the palisade; algal zone about 100 μ thick, of closely coiled filaments, forming ellipsoidal colonies, cells spherical to angular from mutual pressure, *Nostoc* or perhaps *Scytonema;* medulla of loosely woven hyphae 2–3 μ

in diameter, in places more or less parallel as in *Coccocarpia pellita*, especially at the margin of the thallus with about 1 row of agglutinated black thick-walled hyphae functioning as a lower cortex, giving off rhizinae either as single hyphae or as small fascicles of hyphae; sterile.

This species has not been seen fertile and its systematic position is uncertain. It has somewhat the appearance of an isidiose *Malmella physcioides* but differs from the latter in a less well-developed tomentum, a thinner upper cortex, and a thicker algal zone. It differs much more from *Coccocarpia cronia* in the loosely tangled tomentum layer which is present in *Coccocarpia* but closely appressed and formed of longitudinal hyphae, in the loosely tangled medulla (in *Coccocarpia* almost pseudoparenchymatous of periclinal hyphae), and the much less developed lower cortex.

MALMELLA **albida** Dodge, sp. nov.

Type: Costa Rica, Limón, R. Siquirres, 70–200 m., *Dodge, Catt & Thomas 8060.*

Hypothallus crassus, niger, bene evolutus; thallus albidus, cinerascens, laciniatus, di- vel trichotomus aut irregularis, laciniis linearibus, 0.2–0.5 mm. latis, et superficiei marginibusque dense isidiosis, coralloideis, inferne niger, rhizinis nigricantibus; thallus 80–100 μ crassitudine, cortex superior hyphis periclinalibus 5 μ et cellulis pseudoparenchymatibus 20 μ metiens, zona nostocacea (scytonematica?) 60 μ crassitudine, filamentis contortis, cellulis sphericis, 4–5 μ diametro; medulla 40–45 μ crassitudine, hyphis laxis implexis; cortex inferior 8–12 μ crassitudine, hyphis nigris pachydermatibus conglutinatis compactus. Sterilis.

Hypothallus thick, black, well developed; thallus chalk-white, becoming ashy from isidia, laciniate, di- or trichotomous or wholly irregular, laciniae linear, 0.2–0.5 mm. broad, upper surface and margins densely isidiose, coralloid, below black with black rhizinae; thallus 80–100 μ thick, upper cortex about 25 μ thick, the outer 5 μ of periclinal hyphae, below which are about two layers of pseudoparenchymatous cells; the algal layer of *Nostoc* (*Scytonema* ?) about 60 μ thick, of contorted filaments of spherical cells 4–5 μ in diameter; medulla 40–45 μ thick, of loosely tangled hyphae; lower cortex 8–12 μ thick, of thick-walled,

black, conglutinate hyphae; sterile. Known only from a single small collection in Costa Rica.

Limón: R. Siquirres, 70–200 m., *Dodge, Catt & Thomas 8060.*

MALMELLA **Santamariae** Dodge, n. sp.

Type: Costa Rica, Guanacaste, H. Santamaría, 640–680 m., *Dodge & Thomas 8049.*

Hypothallus nullus sed rhizinae coeruleo-nigricantes, dense implexi, ei simulantes; thallus albidus, minute tomentosus, palmatim lobatus, lobis oblongis, 0.5–1 mm. latitudine, 120 μ crassitudine, cortice filamentosa, 15–20 μ crassitudine hyphis pachydermaticis periclinalibus; strato nostocaceo 40 μ crassitudine in medulla gradatim transeunte, rhizinis nigris pachydermaticis 4–5 μ diametro metientibus. Apothecia 0.5–0.75 mm. diametro marginibus crassisimis crenulatis inflexis, disco nigro; amphithecium inferne 140 μ crassitudine superne ad 100 μ attenuatum, cortice 40 μ crassitudine pseudoparenchymatico; parathecium 20–30 μ crassitudine, hyphis tenuibus periclinalibus dense implexis; thecium 140–160 μ altitudine; paraphyses tenues, filiformes, apicibus in gelatina dilabentibus; asci clavati, 12 μ diametro; ascosporae distichae, octonae, 10–11 x 7–8 μ, ellipsoideae, episporiis crassis.

Hypothallus of densely tangled bluish black rhizinae; thallus white, minutely tomentose, palmately lobed, lobes oblong, 0.5–1.0 mm. broad, 120 μ thick; cortex filamentous, 15–20 μ thick, of thick-walled hyphae, algal layer of *Nostoc* gradually merging into the medulla; rhizinae thick-walled, black, 4–5 μ in diameter. Apothecia 0.5–0.75 mm. in diameter, with very thick inrolled margins and blackening disc; amphithecium 140 μ thick below, thinning to 100 μ at the margin, cortex 40 μ thick, pseudoparenchymatous; parathecium 20–30 μ thick, of densely appressed periclinal hyphae not clearly differentiated from the hypothecium; thecium 140–160 μ tall; paraphyses very slender, filiform, degenerating into the dark brown epithecial gel; asci clavate, 12 μ in diameter, 8-spored; ascospores distichous, ellipsoidal, with thick epispore, 10–11 x 7–8 μ.

While eventually this species may be found to have a much wider distribution, it seems fitting that the excellent H. Santamaría, with one of the richest lichen floras known to me, should be

commemorated in botanical literature. The hacienda in turn preserves the name of the national hero of Costa Rica not far from the scene of his famous exploits.

Guanacaste: H. Santamaría, 640–680 m., *Dodge & Thomas 8049.*

MALMELLA **physcioides** (Vainio) Dodge, n. comb.

Erioderma physcioides Vainio, Jour. Bot. Brit. & For. **34**: 70. 1896.

Type: West Indies, St. Vincent, Boxwood, *W. R. Elliott 252.*

Hypothallus sparingly developed, strigose-pannose, greenish black; thallus deep olive buff, radiately subpinnatifid, ultimate lobes 1–1.5 mm. broad, short and convex, surface toward the margin slightly spongy, very minutely verrucose, about 120 μ thick, upper cortex about 40 μ thick, of large thin-walled pseudoparenchyma, the rest of loosely woven hyphae with very small, separate colonies of *Nostoc*, the lower 10 μ with more densely woven, more or less parallel hyphae. Apothecia circular to quite irregular by mutual pressure, crowded, about 1 mm. in diameter, margin concolorous with the thallus, crenulate, disc chestnut; amphithecium about 200 μ thick, the outer 60 μ of tomentum consisting of slender, thin-walled hyphae more or less perpendicular to the surface and somewhat tangled. Within this is the true cortex about 30 μ thick of large-celled pseudoparenchyma, thinning out to a single layer above, and the algal layer of slender periclinal thin-walled hyphae and colonies of *Nostoc* about 100 μ thick below and about 80 μ above; parathecium about 40 μ thick, of densely woven periclinal hyphae, staining very lightly and reaching the margin above; hypothecium deeply staining, 15–20 μ thick, of densely woven hyphae; thecium 100–120 μ tall; paraphyses filiform, 2 μ in diameter, frequently septate, disintegrating into the brownish epithecial gel; asci clavate, 6–8 μ in diameter, 8-spored; ascospores broadly ellipsoidal, 10–12 x 6–8 μ, with a gelified wall, minutely roughened within.

Previously reported only from the type locality. I have previously distributed duplicate material of this species under the name *P. imbricatula* Müll. Arg., which was originally briefly described from southern Brasil. Only a careful study of both types can settle its identity and relationships.

Alajuela: Fraijanes, 1500–1700 m., *Standley & J. Valerio 47651;* S. Isidro de Alajuela, 980–1300 m., *Dodge, J. Valerio & Thomas 4883.*

Guanacaste: H. Santamaría, 640–780 m., *Dodge & Thomas 8034, 8035.*

Malmella **caesiocinerea** (Vainio) Dodge, n. comb.

Pannaria rubiginosa f. *cinerascens* Vainio, Jour. Bot. Brit. & For. **34**: 70 [10]. 1896, non Nyl.

Pannaria rubiginosa f. *caesiocinerea* Vainio, Acta Soc. Sci. Fenn. **A6**[7]: 102–103. 1915.

Type: not stated although several specimens mentioned. *P. rubiginosa* f. *cinerascens* Vain. based on Antilles, St. Vincent, Mt. St. Andrews, 320 m., *Elliott 15*. The following description is based on Costa Rican material.

Rhizinae bluish black, highly developed and tangled; thallus buffy brown in the center, lobes dark olive with a white pruina about the margins, tomentose, lobes rounded, 1–4 mm. broad, sinuate, incised, the lobules oblong, about 0.5 mm. broad, convex, margins thick, inflexed below, 200 μ thick, the upper cortex a palisade of thick-walled hyphae about 20 μ thick, homoeomerous, with coiled filaments of *Nostoc* distributed throughout a very loosely tangled layer of hyphae, fraying out below without lower cortex. Apothecia up to about 0.5 mm. in diameter, margin very thick and inrolled, white, crenulate, disc black; amphithecium thick, continuous with the thallus, about 140 μ thick, with cortex about 60 μ thick below and with algae much more abundant and closely tangled than in the thallus; parathecium thin, filamentous, 20 μ thick, not differentiated from the hypothecium; thecium 80–90 μ tall; paraphyses filiform, septate, ending in the hypothecial gel which is deep brown and deeply staining; asci clavate, about 20 μ in diameter, 8-spored; ascospores distichous, ellipsoidal, 11–12 x 6–7 μ, with a gelified epispore about 2 μ thick, sometimes appearing 2-celled or even 4-celled with 2 transverse septa and 1 longitudinal septum in the middle, probably due to oil globules or other highly refractive substances, as other spores in the same ascus seem to be definitely unicellular.

This species suggests a close relationship with *Lepidocollema* but the cortex is much better developed and the apothecium is lecanorine.

Guanacaste: H. Santamaría, 640–680 m., *Dodge & Thomas 8033*.

PARMELIELLA Müll. Arg.

Parmeliella Müll. Arg., Mem. Soc. Phys. Hist. Nat. Genève **16**: 376. 1862.

Type species: *P. triptophylla* (Ach.) Müll. Arg. [*P. corallinoides* (Hoffm.) Zahlbr.].

Thallus squamose, becoming elongate and subfoliose at the margins with a well-developed dark hypothallus, algae *Nostoc;* upper cortex of thick-walled pseudoparenchyma; medulla loosely woven; lower cortex an appressed layer of rhizinae. Apothecia superficial; amphithecium absent; parathecium pseudoparenchymatous; asci 8-spored; spores hyaline, unicellular, elongate-ellipsoidal with thin walls; spermatia short, straight.

This genus seems world-wide in its distribution, perhaps predominantly of the southern hemisphere but widespread in the North Temperate zone and in the tropics, preferring the higher elevations. Only *P. pannosa* has yet been found in Costa Rica. The other species have been incompletely described but have characters which should make them recognizable if found.

KEY TO THE TROPICAL AMERICAN SPECIES OF PARMELIELLA

Hypothallus indistinct, thallus glauco-cinereous or pale, squamules confluent, crenate, granulate; spores small, 8–9 x 4–6 μ; Chile.... *P. microphylloides*

Hypothallus forming a black margin about the thallus.

Hypothallus very thin, blue green; spores 15–19(–21) x 6.5–8 μ; surface smooth; thecium 100 μ thick; Chile..... *P. nigrocincta*

Hypothallus blue black, densely woven, cortex a single layer of cells; spores 11–17 x 5–8 μ; thecium 110 μ thick; Mexico..... *P. miradorensis*

Hypothallus brownish black, densely woven and thick, cortex several layers of pseudoparenchyma; spores 12–15(–18) x 6–8 μ; thecium 120–140 μ thick..... *P. pannosa*

Hypothallus very thick; apothecia 1–1.5 mm. in diam.; spores 16–25 x 8–10 μ; Bolivia..... *P. nigrocincta* var. *Weddellii*

PARMELIELLA PANNOSA (Sw.) Müll. Arg., Flora **64**: 86. 1881.

Lichen pannosus Sw., Nova Gen. Sp. Pl. Prodr. 146. 1788; Fl. Ind. Occ. **3**: 1888. 1806.

Type: Jamaica, *O. Swartz.*

Hypothallus brownish black, extending 1–2 mm. beyond the thallus; thallus tawny olive to isabelline, irregularly somewhat pinnatifidly divided in the margin, the central portion of the thallus becoming appressed microphylline and even somewhat isidioid, margins of lobes somewhat lighter in color, otherwise smooth above; thallus about 100 μ thick, the upper cortex 40 μ thick, of large thick-walled spherical cells forming a pseudoparenchyma, algal zone thin, about 20 μ thick, of small somewhat

confluent colonies, medulla about 40 μ thick, of large thin-walled, loosely woven hyphae, the lower cortex 10–12 μ thick, of conglutinate, black, thick-walled hyphae. Apothecia abundant, about 1 mm. in diameter, round to irregular in shape, the margin lighter colored when young, appearing to be thalline but not so, darkening in age but always slightly lighter than the disc and not exceeding the epithecium in height, disc rufous; amphithecium absent; parathecium 100–120 μ thick, pseudoparenchymatous, of large thick-walled cells extending to the top of the thecium; hypothecium about 40 μ thick, of densely woven hyphae, deeply staining; thecium 120–140 μ thick; paraphyses branched above, 1.5–2 μ in diameter, with thin walls; asci clavate, about 15 μ in diameter, 8-spored; ascospores irregularly distichous, ellipsoidal, 12–15 x 6–8 μ, epispore thick.

This species seems to be the commonest one in tropical America, in our area confined largely to higher elevations than *Pannaria*, being most abundant above 650 m., but rarely found at lower elevations.

Without locality: *Ørsted 8.*

Limón: Waldeck, *Dodge 7403.*

Cartago: R. Pejivalle, 600–750 m., *Dodge & Thomas 4341, 8038;* Cartago, 1200–1500 m., *C. Werckle* (Mus. Nac. 17218).

San José: Sta. María de Dota, 1500–1800 m., *Standley & J. Valerio 43394, 44057, 43444, 43446, 43472;* La Hondura, 1300–1700 m., *Standley 36458b.*

Heredia: Barba, El Gallito, *R. Torres 150;* C. Central de Zurquí, 1600–1700 m., *Dodge, J. Valerio & Thomas 8039.*

Alajuela: S. Ramón, La Palma, 1100 m., *Brenes 54;* Piedades, 900 m., *Brenes 380;* Alto Calera, 750–800 m., *Brenes 265.*

Guanacaste: H. Santamaría, 680–780 m., *Dodge & Thomas 6892;* H. Granadilla, 500–600 m., *Dodge & Thomas 6746.*

Var. **coralloidea** Dodge, var. nov.

Type: Costa Rica, Heredia, C. Zurquí, 1600–1700 m., *Dodge, J. Valerio & Thomas 8061.*

Thallus iteratim laciniatus sed non microphyllinus, isidiis coralloideis marginalibus rare sparsis, obscurior, 140 μ crassitudine, cortex superior 25–30 μ crassitudine, duobus seriebus cellularum, serie superiore fusca, inferiore hyalina; stratum nostocaceum ad 60 μ crassitudine, filamentis dense contextum; medulla hyphis magnis laxe implexa; cortex inferior ad 12 μ crassitudine, hyphis nigris, pachydermaticis, conglutinatis, compactus; sterilis.

Thallus repeatedly laciniate but not microphylline, isidia coralloid, mostly marginal, rarely scattered over the surface, darker, 140 μ thick, the upper cortex 25–30 μ thick, of two layers of thick-walled cells, the outer layer fuscous, the inner hyaline; the algal layer about 60 μ thick, of densely woven filaments of *Nostoc;* medulla of large loosely tangled hyphae; lower cortex about 12 μ thick of large black, thick-walled hyphae; sterile.

Heredia: C. Central de Zurquí, 1600–1700 m., *Dodge, J. Valerio & Thomas 8061.*

ERIODERMA Fée

ERIODERMA Fée, Essai Cryptog. Ecorc. Offic. 146. 1824.

Type species: *Erioderma polycarpum* Fée.

Thallus foliose, attached to the substrate by either marginal rhizinae or those from the under surface; tomentum highly developed, cortex pseudoparenchymatous, formed by a palisade layer, the algal zone of short chains of *Scytonema* with thin sheaths or *Nostoc;* medulla loosely woven of thin-walled hyphae; no lower cortex, the lower surface somewhat veined and in some species with a dense covering of rhizinae. Apothecia marginal or superficial, peltate, constricted at the base; amphithecium absent; parathecium well developed, of large thick-walled cells, pseudoparenchymatous, with a loosely woven medulla; asci 8-spored; spores hyaline, unicellular, ellipsoidal, occasionally fusiform or subspherical. Spermagonia marginal, small, blackish warts; spermatiophores septate; spermatia short, cylindric, straight.

KEY TO THE TROPICAL AMERICAN SPECIES OF ERIODERMA

Dense blackish fuscous tomentum below, suggesting hypothallus.
  Lobes narrow, deeply sinuate divided margins.....*E. Wrightii*
  Lobes broader, margin with sub-granulate caesious soredia below, and white-villose.....*E. limbatum*
Nearly nude and veined, white below, although rhizinae bluish black or white, verruculose and villose above; Brasil.....*E. verruculosum*
Sulphur yellow below with white veins and rhizoids, coarsely short-pilose above; apothecia up to 0.8 mm. in diam.; Jamaica.....*E. microcarpa*
Bluish white with fleshy white veins and rhizoids, hairs tufted above, giving an irpiciform appearance when dry; apothecia 3–4 mm. in diam.; Brasil.....*E. pulchrum*
White below; thallus isidiose; apothecial margin isidiose, ashy virescent above; Brasil.....*E. Leylandi*

ERIODERMA WRIGHTII Tuck., Amer. Jour. Arts & Sci. II, **25:** 423. 1858.

Type: Cuba, top of Loma del Gato, *C. Wright.*

Hypothallus none, but dense brownish-black rhizinae clothe the under surface to within 3 mm. of the margin which is arachnoid-tomentose, white; thallus deep olive buff to citrine drab, sinuate-lobed, upper surface tomentose, margins with tufts of dark brown rhizinae, thallus about 120 μ thick exclusive of tomentum on upper surface and rhizinae below; cortex pseudoparenchymatous, 30–40 μ thick, of large cells, algal layer very thin, 10–15 μ thick, of densely coiled filaments of *Scytonema;* medulla very loosely woven, similar to the tomentum above; no lower cortex. Apothecia marginal with white densely pilose exciple and black disc; amphithecium absent; parathecium 200 μ thick, corticate with pseudoparenchyma about 25 μ thick below, a little less above, formed from a palisade of hyphae perpendicular to the surface, the cortex covered with tomentum as in the thallus, the rest of the parathecium of large, loosely woven hyphae similar to the medulla but somewhat more closely woven; hypothecium not well differentiated from the parathecium, rather more densely woven; thecium about 120 μ tall; paraphyses filiform, slender, branched above, ending in dense brown epithecial gel; asci clavate, 8-spored, 14–16 μ in diameter; ascospores distichous, 12–14 x 6–7 μ, ellipsoidal, with a thick epispore.

This very distinct species resembles the Peltigeraceae in habit much more than the Pannariaceae, but it has the typical unicellular spore with the thick epispore of the latter family. It seems rather rare in tropical America but it is quite abundant in the few localities where it has been found. In Costa Rica it occurs from 680 to 1100 m.

Alajuela: La Palma de S. Ramón, 1100 m., *Brenes 50.*
Guanacaste: H. Santamaría, 680–780 m., *Dodge & Thomas 6920, 6982.*

ERIODERMA **limbatum** (Nyl.) Dodge, n. comb.

Erioderma Wrightii var. *limbatum* Nyl., Flora **52**: 119. 1869.

Type: Brasil, Rio de Janeiro, Serra dos Orgãos, *Glaziou 2004.*

Thallus cinnamon buff to chamois, lobes rounded-sinuate, slightly excised, 5 mm. broad, margins crenate, thick, upper surface only slightly tomentose, lower surface with margins caesious-sorediate, white; rhizinae greenish black, very dense and thick; thallus about 300 μ thick, upper tomentum not highly

developed; cortex 25–30 μ thick, pseudoparenchymatous from the disintegration of a palisade; algal layer about 60 μ thick, of irregularly tangled filaments of irregular somewhat angular cells about 8 μ in diameter (*Scytonema* ?); medulla about 60 μ thick next the cortex, of rather densely tangled hyphae, gradually becoming looser to form another indefinite layer 120 μ thick, of very large and very loosely tangled hyphae 10–12 μ in diameter, with black hyphae of about the same size forming a more dense mat which serves as a lower cortex. Apothecia 3 mm. in diameter, stipitate, stipe 1–2 mm. tall, margin thin, erect or incurved, densely tomentose without, the hairs tangled, giving a granular appearance, disc chestnut, soon blackening; no amphithecium; parathecium highly developed, cortex a palisade of thick-walled infrequently septate hyphae proliferating to form the tomentum and medulla, 200 μ or more thick below, thinning out at the edge of the thecium so that only the cortex about 100 μ thick surrounds the upper portion of the thecium at the margin; hypothecium not differentiated; thecium about 90 μ tall; paraphyses slender, filiform, apex not enlarged; asci cylindrical, about 8 μ in diameter, 8-spored; ascospores monostichous, at least when found, thick-walled, hyaline, ellipsoidal, 10–12 x 5–6 μ.

This species, from its habit and color, might easily be mistaken for *Sticta Weigelii* or *S. rufa* except for a somewhat duller surface, but is distinguishable on the color and density of the rhizinae and the lack of cyphellae. Known from Costa Rica from a single collection with scant data by *Brenes . . . a*, probably from the region of S. Ramón in Alajuela Province.

COCCOCARPIA Pers. ap. Gaudich.

Coccocarpia Pers. ap. Gaudich. in Freycinet, Voy. Uranie, Bot. 206. 1826.

Type species: *Coccocarpia molybdaea* Pers. ap. Gaudich.

Thallus squamose to foliose, with dark or light-colored rhizinae, corticate on both surfaces, upper surface pseudoparenchymatous from thin-walled large hyphae parallel to the surface, without tomentum; algal layer of coiled chains of *Scytonema* in thin sheaths; medulla of thin-walled somewhat conglutinate hyphae not sharply differentiated from the lower cortex, of more or less

septate hyphae running parallel to the surface. Apothecia superficial, sessile or somewhat constricted below; amphithecium absent; parathecium corticate with large-celled pseudoparenchyma, within, of large septate periclinal hyphae, medulla lacking; hypothecium either light or dark; asci 8-spored; ascospores hyaline, unicellular, spherical to ellipsoidal-fusiform, thin-walled. Spermagonia in warts on the thallus, spermatiophores frequently septate; spermatia straight, elongate-cylindric.

This widespread tropical genus has two very widespread species or species groups and a number of rare and seemingly localized species scattered over the tropical regions.

KEY TO THE TROPICAL AMERICAN SPECIES OF COCCOCARPIA

Under side reticulate, black-fibrillose; lobes obtuse, ciliate, margin of apothecia crenulate, disc yellow ..... *C. portoricensis*
Under side densely black-tomentose, forming a hypothallus.
Lobes bipinnately divided, linear, ciliate, aeruginous-green ..... *C. subtilis*
Lobes cuneate to flabelliform, bluish to lead color.
Thallus isidiose or microphylline in the center ..... *C. cronia*
Thallus not reddish ferruginous within.
Isidia concolorous, laciniae cuneate, 1.5–10 mm. broad.
Laciniae 5–10 mm. broad, isidia applanate to microphylline ..... v. *prolificans*
Laciniae 1.5–5 mm. broad, isidia terete; apothecia yellow or rufo-fuscous, exciple white-ciliate ..... v. *isidiophylla*
Isidia usually darker at the tips and blackening, laciniae narrower and sublinear.
Laciniae 1–1.5 mm. broad; hypothallus little developed; apothecia fuscous, blackening, exciple white-ciliate; Brasil .. v. *camporum*
Laciniae 0.5–3.0 mm. broad, narrow granulose to lobulate in the center; apothecia black; hypothallus well developed .... v. *granulosa*
Thallus reddish ferruginous within; Cuba ..... v. *erythrocardia*
Thallus not isidiose in center ..... *C. pellita*
Laciniae cuneate or broad-spathulate.
Hypothallus very highly developed; apothecia yellow to tawny when young ..... v. *pannosa*
Hypothallus present but much less well developed.
Apothecia black from the first, laciniae broad ..... v. *parmelioides*
Apothecia yellow, rufous, or fuscous.
Thallus very thin, lobes small, approaching microphylline states of *C. cronia* v. *prolificans;* apothecia finally darkening ..... v. *lividorufa*
Thallus thicker, lobes broad, 4–8 mm.; apothecia not darkening, reddish ..... v. *pyrrhichocarpa*
Laciniae sublinear, up to 4 mm. broad, short, rounded.
Apothecia black; Jamaica ..... v. *genuina*
Apothecia fuscous, ciliate ..... v. *strigosa*

Under side of thallus white with black-fibrillose margins, laciniae lacerate-imbricate.....*C. fibrillosa*
Under side of thallus white, rhizinae white.
Thallus isidiose.
Laciniae very narrow, 0.1–0.2 mm. broad, adnate.....*C. dominguensis*
Laciniae broad and flabelliform, up to 10 mm. broad.....*C. albida*
Thallus not isidiose.
Laciniae 150–200 μ broad, loosely adnate, bluish; rhizinae penicilloid, projecting beyond margin, dichotomous.....*C. tenuissima*
Laciniae 250 μ broad, tips ascending, bluish; long white rhizinae, palmate with lacinulae pinnatifid; apothecia carneous, rigidly white-ciliate.....*C. elegans*
Laciniae broader.
Thallus greenish blue, rhizinae rigid, white; flattened apothecia cerine-flavous.....*C. epiphylla*
Thallus lead-color or slightly bluish, crenate-squamulose, subimbricate, pterygoid; apothecia testaceous, white, ciliate.....*C. asterella*

COCCOCARPIA PELLITA (Ach.) Müll. Arg., Flora **65**: 320. 1882.

Parmelia pellita Ach., Lichenog. Univ. 468. 1810, Sw., Lich. Amer. 7, *pl. 6*. 1811.

Type: West Indies, *O. Swartz*.

Laciniae of thallus rather narrow, about 4 mm. broad, pinnatifidly incised, either discrete or approximate, smooth above; hypothallus well developed and extending beyond the thallus. Apothecia deep fuscous.

This species seems widely distributed and very variable in the tropics. Only a monographic study by one who has access to the types of all the proposed varieties as well as considerable experience in the field in the principal floristic areas of the tropics can finally decide the validity of the proposed varieties and forms and provide adequate descriptions so that others may recognize them. Nylander, Müller Argau, Hue, Vainio, and Malme have each attempted more or less elaborate revisions but none seems wholly satisfactory. While probably artificial the separation of *C. cronia* for all the isidiose varieties of this species is relatively easy to apply, and has been followed here.

Var. PARMELIOIDES (Hook. ap. Kunth) Müll. Arg., Flora **65**: 320. 1882.

Lecidea parmelioides Hook. ap. Kunth, Syn. Pl. Aequinoct. Orb. Nov. **1**: 15. 1822.

Type: Colombia, Cumana, Bordones, and Nueva Barcelona, *Humboldt & Bonpland*.

Thallus light olive gray, surface smooth, lobes cuneate, about 4 mm. wide, sometimes once-cleft, closely appressed to the substrate, held by a few dark brown to black rhizinae composed of fascicles of hyphae, about 200 μ thick; upper cortex about 25 μ thick, the outer 5 μ formed from the disintegration of a very thin layer of periclinal hyphae, the rest pseudoparenchymatous from the shifting of cells in a palisade layer, cells 10–12 μ in diameter, rather thick-walled; algal layer a palisade of hyphae and filaments of *Scytonema* about 100–120 μ thick; medulla about 40 μ thick, of large septate periclinal hyphae 5–8 μ in diameter; lower cortex of dark, periclinal conglutinate thick-walled hyphae 3–4 μ in diameter. Apothecia immarginate, appressed and more or less adnate, 2 mm. or more in diameter, disc black; no amphithecium; parathecium of periclinal thick-walled hyphae, giving a pseudoparenchymatous appearance, 100–120 μ thick, thinning out to about 20 μ at the edge; hypothecium not differentiated from the parathecium; thecium about 160 μ tall; paraphyses 2–3 μ in diameter, frequently septate, clavate tips black, about 4 μ in diameter; asci cylindric to somewhat clavate, 8-spored, about 60 x 8–12 μ; ascospores monostichous at first, gradually becoming distichous, ellipsoidal with acute ends, very thick-walled, unicellular, often with some deeply staining material in the middle with a slight thickening of the cell wall at that point, giving the appearance of a two-celled polarilocular spore with a wide isthmus, occasionally two other constrictions near the tip slightly suggesting a 4-celled condition, 11–12(–16) x 6–7 μ.

This variety seems widespread in the lower elevations although in absence of apothecia it is practically indistinguishable from var. *pyrrhichocarpa*. In Costa Rica var. *parmelioides* occurs in suitable habitats up to about 800 m., while var. *pyrrhichocarpa* occurs from 700 to 1340 m., but seen from Brasil up to 2000 m. in the state of São Paulo.

Limón: Waldeck, 40 m., *Dodge 7402*.

Guanacaste: H. Santamaría, 680–780 m., *Dodge & Thomas 4709;* C. San José de Líbano, 500–960 m., *Dodge, Hanckel & Thomas 6684;* R. San José, 460–480 m., *Dodge & Thomas 6581*.

Puntarenas: Osa, Puerto Jiménez, *Brenes 825a;* R. Sándalo, *Dodge 7742*.

Var. PYRRHICHOCARPA Hue, Bull. Soc. Bot. France **48**: lx. 1901 [1902].

Var. *smaragdina* Müll. Arg., Flora **65**: 320. 1882, excl. syn. quoad specimina americana; Vainio, Étude Lich. Brésil **1**: 210. 1890; Malme, Ark. f. Bot. **20**^{3}: 19. 1924.

Type: Brasil, São Paulo, near São Paulo, *Azevedo Sampaio.*

Thallus between yellowish glaucous and light mineral gray, lobing of thallus similar to var. *parmelioides* but much more irregular and lobes rather smaller, smooth above, 150–160 μ thick, upper portion of cortex 4–6 μ thick, of longitudinal hyphae soon disintegrating and disappearing, leaving a palisade 25–30 μ thick, which in turn becomes a somewhat irregular large-celled pseudoparenchyma; the algal layer 40–50 μ thick, of loosely coiled and disintegrating filaments, perhaps *Scytonema*, but the cells rounding up and suggesting *Nostoc*, about 8 μ in diameter, the medulla 50 μ thick, of large septate periclinal hyphae, somewhat less compact than in var. *parmelioides*, the lower cortex about 25 μ thick, of black somewhat smaller conglutinate hyphae; rhizinae much more abundant, smaller conglutinate fascicles of hyphae similar to those of the lower cortex. Apothecia irregular, 3–3.5 mm. in diameter, carnelian red to vinaceous rufous, only slightly darkening, never dark brown or black, immarginate; amphithecium absent; parathecium adnate to the upper cortex, about 100 μ thick, of large periclinal hyphae appearing almost pseudoparenchymatous at times; thecium 60 μ tall; paraphyses 2–3 μ in diameter, filiform, apex not enlarged; asci clavate, about 12 μ in diameter; ascospores 10–14 x 4–5 μ.

This variety is occasional at the medium elevations of the temperate zone in Costa Rica, ranging from 700 to 1400 m. It should not be confused with *C. smaragdina* Pers. or *C. molybdaea* Pers., both from the Old World tropics.

Cartago: R. Birrís near Santiago, 920–1340 m., *Dodge 4708, 8050;* Cartago, *C. Werckle* (Mus. Nac. 172186 p. min. p.).

Alajuela: Santiago de S. Ramón, 1000 m., *Brenes 238.*

Guanacaste: H. Santamaría, 680–780 m., *Dodge & Thomas 6813.*

Var. STRIGOSA Müll. Arg., Flora **65**: 326. 1882.

Coccocarpia molybdaea v. *cronia* Nyl., Acta Soc. Sci. Fenn. **7**: 441. 1863, non Tuck.

Type: Colombia, Cune, 1200 m., *Lindig 2663 p. p.*

Thallus between pale olive buff and yellowish glaucous, lobes cuneate but narrower than in v. *parmelioides*, slightly incised,

smooth and shining above, thallus about 100 μ thick, outermost layer of hyphae about 4 μ thick, soon disintegrating, upper cortex pseudoparenchymatous, 10–12 μ thick, of about two layers of pseudoparenchymatous cells not part of a palisade, algal layer 32–40 μ thick, of curved hyphae in a disintegrating palisade (intermediate between v. *parmelioides* and v. *pyrrhichocarpa*), medulla 25–30 μ thick, of large periclinal hyphae, lower cortex 12–15 μ thick, of large black periclinal hyphae, closely agglutinated rhizinae abundant but hyphae less fasciculate. Apothecia about 2 mm. broad, ochraceous tawny at first, becoming Prout's brown or darker, immarginate; no amphithecium; parathecium about 100 μ thick, of large periclinal hyphae, at the margins many hyphae projecting beyond the general level as stiff hairs; hypothecium not differentiated and not deeply staining; thecium about 40 μ tall; paraphyses filiform with acuminate tips which extend 1–2 μ above the epithecial gel; asci clavate, 30 x 8 μ, with 8 spores; ascospores fusiform-ellipsoidal, 8 x 4 μ, thick-walled, probably still immature.

In the only collection from Costa Rica the thallus may not be quite mature, as no mature ascospores were found, hence the measurements may be a little small. Reported elsewhere only from Colombia.

Cartago: R. Birrís, 920–1100 m., *Dodge 8051*.

COCCOCARPIA CRONIA (Tuck.) Vainio, Ann. Acad. Sci. Fenn. A6[7]: 103. 1915.

Parmelia cronia Tuck., Proc. Amer. Acad. Arts & Sci. 1: 228. 1848; Syn. Lich. New England, 36. 1848.

Coccocarpia molybdaea v. *cronia* Nyl., Syn. Meth. Lich. 2: 41. 1863.

Coccocarpia pellita v. *cronia* Müll. Arg., Flora 65: 321. 1882.

Coccocarpia parmelioides v. *cronia* Hue, Bull. Soc. Bot. France 48: lx. 1901 [1902].

Type: United States, Massachusetts, Lynn Hills, and West Cambridge, on mossy rocks, *Tuckerman*.

Thallus mineral gray or darker, lobes rounded or short, broad-linear, more or less appressed, growing over mosses, about 130–140 μ thick; upper cortex 15–20 μ thick of large thin-walled hyphae often appearing pseudoparenchymatous; algal zone

30–40 μ thick, with loosely woven hyphae in which are imbedded solid little groups 15–20 μ in diameter of algal cells of closely coiled *Scytonema;* medulla 30–40 μ thick, partly of close periclinal hyphae, partly more loosely woven, the lower cortex of two layers of black thick-walled hyphae giving rise to rhizinae, sterile; isidia terete, concolorous, or somewhat darkened at the tips. Tuckerman noted on a specimen from Alabama, "spores 9–12 x 3–5 μ, 2-celled?"

Var. LIVIDORUFA (Mey. & Fw.) Zahlbr., Cat. Lich. Univ. **3**: 287. 1925.

Parmelia lividorufa Mey. & Fw., Nova Acta Acad. Leopold. Carolin. **19**: Suppl. 222, *pl. 4, f. 2.* 1843.

Coccocarpia molybdaea var. *tenuior* Nyl., ap. Krmplhbr., Flora **59**: 76. 1876 (nom. nud.).

Coccocarpia pellita var. *tenuior* Müll. Arg., Flora **65**: 321. 1882.

Type: Brasil, Rio de Janeiro, *Meyen* (Bot. Mus. Berlin), type of var. *tenuior;* same locality, *Glaziou 2026* (Univ. Genève).

Thallus mineral gray, lobes small, mostly about 1 mm. broad, quite irregular, smooth, about 100 μ thick, structure as in the previous variety. Apothecia immarginate, irregular, convex, disc snuff brown to bister; amphithecium absent; parathecium 130–140 μ thick; hypothecium not differentiated; thecium 80–85 μ tall; paraphyses filiform, ending in the epithecial gel; asci 8-spored, clavate to cylindrical, 12 μ in diameter, ascospores 11–12 x 5–6 μ, ellipsoidal, thick-walled, distichous.

In this variety the whole thallus approaches a microphylline squamose state. Evidently a lowland variety seen also from British Honduras and Nicaragua.

Limón: R. Siquirres, 70–200 m., *Dodge, Catt & Thomas 8058.*
Alajuela: La Palma de S. Ramón, 1100 m., *Brenes 43.*
Guanacaste: Tilarán, 650–690 m., *Dodge & Thomas 6557.*

Var. **isidiophylla** (Müll. Arg.) Dodge, comb. nov.

Coccocarpia pellita v. *isidiophylla* Müll. Arg., Flora **65**: 321. 1882.

Coccocarpia pellita v. *cronia* Vainio, Étude Lich. Brésil **1**: 209. 1890, et auct. recentior., non Tuck.

Coccocarpia cronia v. *primaria* Vainio, Ann. Acad. Sci. Fenn. **A6**[7]: 103. 1915.

Type: Brasil, Rio de Janeiro, *Glaziou 2025.*

Thallus mineral gray or darker, laciniate, flabelliform or cuneate with rounded margin about 4 mm. broad, sometimes somewhat incised along the margin, upper surface isidiose with either simple or slightly branched isidia which are confined to the central portion of the thallus and to a lesser extent of the lobes; upper cortex of large thin-walled hyphae perpendicular to the margins of the lobes, up to about 20 μ thick, closely septate; algal zone 60 μ thick of filaments of *Scytonema* about 12 μ thick with cells about 8 μ in diameter, in the lower portion the algae less crowded, leaving some air spaces, medulla not differentiated from the algal zone; lower cortex 30 μ thick, of conglutinate, black, thick-walled hyphae, giving rise to scattered rhizinae as well as penicillate fascicles of hyphae. Apothecia yellowish-rufous, becoming fuscous, somewhat lobulate, immarginate, adnate; parathecium 55–60 μ in the center, thinning out at the margin, pseudoparenchymatous, of large somewhat periclinal hyphae; hypothecium not differentiated, about 60 μ thick; paraphyses 2–3 μ in diameter, filiform, with swollen tips in the very dark epithecial gel; asci clavate, 8-spored, 8–10 μ in diameter; ascospores distichous, fusiform, immature.

This species is close to *C. pellita* and perhaps should be considered a variety of that species. It has approximately the same distribution, reaching from sea level to about 1700 m. in Costa Rica, 2700 m. in Perú.

Cartago: Aguacaliente, 1240–1460 m., *Dodge & Thomas 7087.*

Heredia: C. Central de Zurquí, 1600–1700 m., *Dodge, J. Valerio & Thomas 6087;* C. de las Caricias, 2000–2400 m., *Standley & J. Valerio 55282.*

Alajuela: La Palma de S. Ramón, 1100 m., *Brenes* (fertile).

Puntarenas: Osa, between R. Sándalo and R. Tigre, 1–2 m., *Dodge 8057.*

Var. **prolificans** (Malme) Dodge, n. comb.

Coccocarpia pellita var. *prolificans* Malme, Ark. f. Bot. **20**^{3}: 19. 1924.

Type: Brasil, Rio de Janeiro, *Regnell 65.*

Thallus mineral gray or darker, lobes flabelliform, somewhat zonate, 5–10 mm. wide, margin rounded, more or less lobulate along the incised portions between lobes, upper surface lobulate and more or less microphylline, lobules concolorous, about 70 μ thick, upper cortex about 16 μ thick, of large thin-walled longitudinal hyphae; algal zone about 30 μ thick, of a loose palisade

of *Scytonema* filaments, medulla about 20 μ thick, of large thin-walled periclinal hyphae with some air spaces; lower cortex about 15 μ thick, of thick-walled black hyphae, giving off fascicles of rhizinal hyphae. Sterile.

This variety is rather common at elevations from 700 to 1400 m. in Costa Rica.

Cartago: R. Birrís, 1220–1340 m., *Dodge & Thomas 4710.*

Alajuela: La Palma de S. Ramón, 1250 m., *Brenes 38a;* Piedades de S. Ramón, 900 m., *Brenes 385.*

Guanacaste: H. Santamaría, 680–780 m., *Dodge & Thomas 6987, 6999.*

Var. **granulosa** (Müll. Arg.) Dodge, comb. nov.

Coccocarpia pellita var. *granulosa* Müll. Arg., Flora **65**: 322. 1882.

Type: Brasil, São Paulo, Apiahy, *Puiggari 244,* and Colombia, Bogotá, 2700 m., *Lindig 2538.*

Thallus thicker, greenish-glaucous-blue or darker, lobes up to 3 mm. broad, the larger lobes more or less incised, obovoid to almost linear, soon blackish granular to almost isidiose in the center, about 140 μ thick, upper cortex a single layer of longitudinal hyphae 4–5 μ in diameter, algal zone of *Scytonema* filaments 40–50 μ thick, forming a palisade interspersed with occasional rows of fungal cells, medulla of large, septate, periclinal hyphae about 60 μ thick, the lower cortex 6–8 μ thick, of thick-walled dark hyphae, not well developed. Apothecia less adnate and somewhat isidiose; no amphithecium; parathecium 150 μ thick, pseudoparenchymatous, some of the outer layer of cells growing downward to form a ciliate to tomentose surface below; hypothecium not differentiated, thecium about 60 μ tall; paraphyses filiform, tips not enlarged, ending in a dark brown epithecial gel; asci clavate, rather immature.

This variety as here defined includes some of Malme's var. *isidiosa* and some material may have been distributed under that name. In Costa Rica this variety is widespread in the temperate regions from 100 m. to 1500 m.

Limón: R. Siquirres, 70–200 m., *Dodge, Catt & Thomas 5595.*

Cartago: Santiago, 1140–1180 m., *Dodge 4638, 8054;* C. Carpintera, 1320–1500 m., *Dodge & Thomas 4763;* Cartago, *C. Wercklé* (Mus. Nac. 17202).

San José: Turrúcares, 540–600 m., *Dodge & Thomas 8056;* R. Virilla below El Brazil, *Dodge 7777.*

Alajuela: C. Pata de Gallo a S. Rafael de S. Ramón, 1200–1250 m., *Brenes 215;* S. Pedro de S. Ramón, 700 m., *Brenes 459.*

Guanacaste: Liberia, 100 m., *Dodge & Thomas 6582;* H. Santamaría, 640–780 m., *Dodge & Thomas 6900, 7007, 8055;* Tilarán, 650–690 m., *Dodge & Thomas 6556, 6559, Standley & J. Valerio 44429, 44520;* H. Granadilla, between R. Las Cañas and R. S. José, *Dodge & Thomas 6719.*

COCCOCARPIA **albida** Dodge, sp. nov.

Type: Costa Rica, Limón, Hamburg, *Dodge & Nevermann 7401.*

Thallus albidus, isidiosus, lobis rotundatis, flabelliformibus, 10 mm. latis, zonatus, inferne albus rhizinis albidis, apicibus rhizinarum nigricantibus, thallus 120–130 μ crassitudine, cortex 15 μ, hyphis longitudinalibus septatis, 5–6 μ diametro; zona scytonematica ad 40 μ crassitudine, filamentis laxis implexis, medulla 40–60 μ crassitudine hyphis periclinalibus compactis; cortex inferior hyphis hyalinis dense compactis; rhizinis albidis fasciculatis penicillatis.

Thallus mineral gray or lighter, densely isidiose in the center, lobes rounded, flabelliform as in *C. pellita* v. *parmelioides*, 10 mm. broad, more or less zonate, below white with white rhizinae, the latter sometimes tipped brownish black; thallus 120–130 μ thick, cortex 15 μ thick, of longitudinal septate hyphae 5–6 μ in diameter; algal zone 40 μ thick, of loosely tangled filaments of *Scytonema*, medulla 40–60 μ thick, of compact periclinal hyphae; the lower cortex of hyaline hyphae more or less agglutinated; rhizinae white, fasciculate, and penicillate.

The lower surface resembles that of *Sticta* sp. quite closely, but cyphellae are absent and the structure is typical of *Coccocarpia*. Perhaps it is an albino variety of *C. cronia* var. *isidiophylla*. It has much broader lobes than *C. dominguensis*.

Limón: Hamburg, 20–30 m., *Dodge & Nevermann 7401.*

COCCOCARPIA ELEGANS Müll. Arg., Flora **64**: 507. 1881.

Type: Brasil, São Paulo, Apiahy, *Puiggari.*

No hypothallus, thallus terre-verte, small, linear, dichotomous (rarely trichotomous at the ultimate branches), larger laciniae 0.1–0.2 mm. broad, ultimate branches much narrower, dull and smooth above, white with white rhizinae below, thallus 80–85 μ thick, upper cortex 8–12 μ thick, decomposed, apparently of slender longitudinal hyphae; algal layer about 40 μ thick, irregular, of loosely woven fungus hyphae supporting short irregular chains of angular to subspherical cells 8–12 μ in diameter, medulla not well differentiated from the algal layer but of very loosely

woven slender hyphae; lower cortex 10–15 μ thick of closely woven hyaline, longitudinal hyphae, the rhizinae 40–50 μ in diameter, large fascicles of hyaline hyphae. Apothecia immarginate, 0.1–0.5 mm. in diameter, chestnut, with long fascicles of hyphae forming stiff bristles about the margin, with stipe about 200 μ in diameter and 40–50 μ tall; no amphithecium; parathecium 60 μ thick, of thick-walled hyphae forming pseudoparenchyma; thecium 60 μ tall; paraphyses slender, filiform, not enlarged at the tips; asci clavate, 4–6 μ in diameter, immature.

The above description is based upon material from Brasil, Rio de Janeiro, *Glaziou 18071*, determined by Müller Argau, who also reports it from Puntarenas, Boruca, 560 m., *Tonduz 5462*. As this is a small epiphyllous species, a large thallus being only 1 cm. in diameter, it is quite possible that it has been overlooked as my epiphyllous material has not yet been examined carefully.

A NEUTRAL (?) STRAIN OF MUCOR SPHAEROSPORUS FROM MISSOURI

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In the caves of the Ranken estate in Missouri, where the atmosphere is moist and the temperature approximately 16° C., was found a segment of a twig matted with a growth of mycelial threads, white in a mass, and hyaline when single. The appearance of the latter was that of many fine root hairs. When transferred to agar substrates, numerous sporangia, sporangioles, oidia, and chlamydospores developed, with no evidence of zygospores. The characteristics and description were similar to those given by Lendner ('08) for *Mucor sphaerosporus* Hagem 1908.

This fungus has been reported chiefly from Europe, and in this country by Waksman ('16) and Gilman and Abbott ('27), as isolations from soil. As far as the author could determine, this is the first report of the above organism from Missouri.

A culture sent to Dr. A. F. Blakeslee at the Carnegie Institution of Washington, Department of Genetics, was tested with strains of presumably the same species, one being a minus isolated by Waksman in 1915, and another a plus strain probably from the Centralstelle, of unknown origin. No zygospores were produced. An attempt was made to obtain imperfect hybridization by treating this strain with some of the strong testers in the above laboratory, but no sexual reaction resulted. Growth on various media produced changes in the amount of mycelium, size and number of sporangia, sporangioles, hyphae, oidia and chlamydospores, and in chromogenesis.

Thanks are due Dr. Blakeslee and Miss Satina of the Carnegie Institution of Washington for their interest and assistance.

BIBLIOGRAPHY

Gilman, J. C., and E. V. Abbott ('27). A summary of the soil fungi. Iowa State Coll. Jour. Sci. **1**: 225–343. 1927.

Lendner, A. ('08). Les Mucorinées de la Suisse. Matériaux pour la flore cryptogamique Suisse **3**[1]: 1–177. *pl. 1–3.* 1908.

Waksman, S. A. ('16). Soil fungi and their activities. Soil Sci. **2**: 103–156. 1916.

A STUDY OF ENDOMYCES CAPSULATUS REWBRIDGE, DODGE AND AYERS: A CAUSATIVE AGENT OF FATAL CEREBROSPINAL MENINGITIS[1]

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INTRODUCTION

The probable taxonomic relationships of the fungi known as yeast-like organisms have for many years attracted much attention and discussion among mycologists, and to a certain extent among medical men. Considerations of their general physiological properties, modes of reproduction, their cytological differentiations, and varied pathogenic abilities were involved. The group comprises several families and many genera and species, with subdivisions of these, which from time to time call forth long and expounding dissertations on the mechanism of reproduction (the perfect stage). The problem of classification is unfortunately a very complex one, since it has been found, at least in the present work and with other closely related fungi which will be considered later, that several forms of development may be present in the same culture. Such phenomena are rather rare but render incorrect any means of classification based on one phase only.

It is well known that the fungi are divided, on the basis of their morphology, cytology, and sexual development, into three major divisions: Phycomycetes, Ascomycetes, and Basidiomycetes, and to these is appended a fourth group, the Fungi Imperfecti, the life history of which is not at all or incompletely known. The yeast-like organisms are considered to constitute a branch of both the Ascomycetes and the Fungi Imperfecti, the former where the production of asci is found, and the latter where no sexual development has been determined. More specifically, the perfect forms are considered to be members of the Endomycetaceae (Gaümann and Dodge, '28; Rewbridge,

[1] An investigation carried out at the Missouri Botanical Garden in the Graduate Laboratory of the Henry Shaw School of Botany of Washington University, and submitted as a thesis in partial fulfillment of the requirements for the degree of doctor of philosophy in the Henry Shaw School of Botany of Washington University.

Dodge, and Ayers, '29; Moore, '33), and Coccidioideaceae (Moore, '32), including such genera as *Endomyces*, *Coccidioides*, *Rhinosporidium*, and *Pseudococcidioides*, while the imperfect forms, as *Monilia*, *Cryptococcus*, *Oidium*, *Mycoderma*, and several others have features which are somewhat similar to the above groups. This of course does not include the true yeasts which are included under the Saccharomycetaceae.

It was because of the imperfect knowledge existing in the field that the author undertook the study of the cytology of *Endomyces capsulatus*, which had been isolated from a case of meningo-encephalitis. During the course of the work it was found desirable to learn more about the phenomena which might be associated with the pathogen, and hence attention was paid to its physiological and other properties. In addition, the occurrence of other infections by closely allied organisms, *E. capsulatus* var. *isabellinus* (Moore, '33), and *E. dermatitidis* (Moore, '33a), have yielded the fungi which have been used in a comparative study, so that what may pertain to *E. capsulatus* has been found to hold true for the latter, with some minor exceptions as chromogenesis and cultural characteristics.

Historical Review

Occasionally debatable points in the field of medical mycology are cleared up by the isolation of some fungus. Such an organism, *E. capsulatus*, was cultured in 1928 from a case of meningitis. The patient was a white, married male, 48 years of age, a furrier by trade. He entered the Boston City Hospital in August, 1928, complaining of having had continuous headaches in the frontal and temporal lobes for a period of three and a half months. His illness began with a hard, tender, slightly movable mass on the medial surface of the lower third of the left thigh, about five months before entry. This mass subsided within four weeks, but a similar mass then appeared beneath the anterior surface of the neck. He developed defects in memory, losing himself on familiar streets, and could not keep track of time. He lost interest and was unable to understand questions or conversation.

The cyst which had been present at the level of the thyroid isthmus between the trachea and anterior border of the sterno-

cleidomastoid muscle was aspirated. Thirty cc. creamy, tenacious, yellow pus was obtained, from which *E. capsulatus* was cultured. Similar organisms were obtained from the pus aspirated from the nodule of the left thigh.

About a month later, the patient's condition became worse. His temperature increased rapidly, as did his pulse and respiration, and he finally died. The clinical diagnosis was mycotic meningo-encephalitis (?), and an X-ray diagnosis showed bilateral pulmonary tuberculosis. It has been the author's experience that an infection of this sort which involves the lungs is often mistaken for tuberculosis, and the diagnosis made here may have been incorrect. The organism from this case has been used in these studies for the greater part, having been kept as a stock culture by alternate transfers on nutrient and Sabouraud's agar.

The second case due to the variety *isabellinus* (pl. 21) occurred in St. Louis and was reported by MacBryde and Thompson. The patient, a white male, 28 years of age, had been a plumber at the time of the appearance of the skin lesions. He was first admitted to the Barnard Free Skin and Cancer Hospital in February, 1930, with a palm-sized annular ulceration on the left forearm just above the wrist, which involved the dorsal surface chiefly and showed clinical signs of blastomycetic dermatitis. Endomycetes were isolated from the pus of this lesion. About 3 cm. to the right of the anus was another lesion about 3 cm. in diameter and involving the anal margin. On the upper part of the right arm was a scar of a lesion which had healed spontaneously. All active lesions were pruriginous and had a foul smell. The patient later, in January, complained of coryza, with a mild cough, pain in the right temporal region, accompanied by a rise in temperature. His headache became universal and intense, posterior to the eyes and extending to the occiput and base of the neck. There was a marked bilateral Kernig reflex action. He had nauseation and vomiting spells. A lumbar puncture gave a cloudy fluid under slight pressure, in which were seen the organisms found in greater abundance with later punctures. At this time he received a large dose of iodine solution which cleared the spinal fluid and decreased the number of budding cells, improving

his health greatly. However, two weeks later he had a relapse, became comatose, and three days before death developed three small subcutaneous abscesses. One was on the right wrist, one on the sacrum, and one on the left side of the chest, and all contained pus and *E. capsulatus* on culture. The patient died forty-six days after the onset of the meningitis symptoms and about three years after the first appearance of the skin lesions.

The morphology and cytology of the organism of this case was found to be the same as the first fungus, having a very light cinnamon color as contrasted with the hyaline or white, in a mass, of *E. capsulatus*, and cultural characteristics similar in detail.

The third organism studied is the one which has gone under many generic names, as *Blastomyces*, *Oidium*, *Saccharomyces*, *Cryptococcus*, and *Mycoderma*, because its life history was incompletely known and the sexual act was not definitely established, or the presence of the ascus and the actual number of spores determined. Historically this fungus is important because it causes a disease known as blastomycosis which has become fairly widespread, both in this country and Europe. The organism studied by the author was isolated from a case which occurred at the Barnard Free Skin and Cancer Hospital of St. Louis and has been dealt with in detail in a previous paper (Moore, '33). Suffice it to say here that *E. dermatitidis*, the organism causing a clinical condition known as blastomycosis, is similar to the above organisms, differing in its chromogenesis, being a dark cinnamon to brown in culture and having a somewhat different cultural reaction, to be mentioned later.

In addition to the above three forms, an organism obtained from a case of the so-called European blastomycosis or generalized torulosis reported by Urbach and Zach ('30) (pl. 19, figs. 13–25) has been studied to a certain extent. The case in brief was that of a 27-year-old shoe-worker who had never left Europe. He entered the University Clinic for Syphilology and Dermatology at Vienna with a swelling of the gums of the lower jaw which was excised, and a half year later he returned with a similar swelling of the gums of the upper jaw. About one year later the patient noticed an abscess-like swelling on the left side of the abdominal region, two months later a similar disorder on

his left thigh, and eight weeks later the same thing was found on his neck. The lesion on his left thigh was aspirated and from it 200 cc. purulent substance was obtained. A short time after that an inflammation of the lungs developed which healed spontaneously, but left him with a cough and vomiting spells. Yeast-like cells were cultured from the purulent secretion of the left thigh.

The disease was clinically identical with syphilis, tuberculosis, leukemia, and several others. Inoculation of an extract of the yeast-like cells (blastomycin) brought forth strong local, focal, and generalized reactions, giving evidence of the presence of blastomycosis.

The lesions were somewhat superficially healed by treatment. The patient later developed high temperatures in the evening, oral pains, deafness, violent coughing at night, and ruby-red expectorations. A tumor of the nasal septum, very suggestive of rhinosporidiosis, had developed and a catarrh of the Eustachian tube as a result of the tumor. There was an ulceration of the right tonsil and other lesions close by, and finally a heavy exudate in his lungs. Treatment gave him relief temporarily, but he returned to the clinic a short time later with a continuous headache, high temperature, and rapid spread of the mouth lesions. Paralysis of the optic musculature set in, with unconsciousness, and death ensued.

The organism from this case has been cultured and subcultured and as yet has shown no final stage as is present in the three above forms. However, some organisms require a long time before the perfect stage is obtained. Furthermore, the histological and pathological condition of the patient as investigated by Chiari ('30), shows the identical tissue reaction present in cases of *E. dermatitidis*.

With this brief review of the history of the organisms investigated, let us now turn to a study of *E. capsulatus*.

Technique

E. capsulatus was kept growing by subculturing on nutrient and Sabouraud's agar as a stock culture. In the work done here, the organism was grown on nutrient agar, a product of the

Digestive Ferments Co., at pH 6.8. In cases where an abundant growth was necessary, with a thick mycelium, Sabouraud's agar, pH 5.6, was employed.

For studying the cytology with regard to the nuclear changes in the sexual act, glycerine agar (beef extract agar plus 6 per cent glycerine, pH 7.1) was used. This medium gave the greatest number of asci in culture, in addition to a thick growth and many important diagnostic features, as chlamydospores, conidia, racquet mycelium, etc. Cultures on nutrient agar and Sabouraud's agar were also used, but the cells were not so good for details as on the above medium.

The cultures were fixed with a number of agents. Flemming's stronger solution shrunk the material rather noticeably, but the weaker did not give very good results either. Bouin's picro-formalin solution, which has been advocated by Kater ('27) and other cytologists working with yeasts, was of no value in this work. Benda's fluid, which is a modification of Flemming's stronger solution, caused the fungus to have too great an affinity for the stains, and inasmuch as it is difficult to destain the organism without causing some sort of damage, it had to be used with caution. Hermann's fluid, which is probably the most expensive of the fixing agents, was also used. This is a variation of Flemming's fluid, the chromic acid being replaced by platinic chloride, and although not particularly recommended by Chamberlain ('32), the author has found it to give the best results. Various other fixing agents were employed, but the results were not worthy of note.

The embedding proved to be a problem because the material had to be fixed and embedded while on the agar. Paraffin was used at first, but in the glycerine agar cultures it would not adhere to the agar substrate and sectioning could not be done without damage to the material. Gradual and repeated changes of paraffin did not remedy the condition. A medium was then tried which contained very little protein and carbohydrate, but no suitable growth could be obtained. Finally the cultures were embedded in Dupont parlodion, using the Jeffrey technique. This method has been recently outlined by Wetmore ('32) and with some modification was applied here. It consists in fixing

the material, in this case either with Hermann's or Benda's fluid, pumping and dehydrating, then passing it through an intermediate stage of ether-alcohol, and then through a series of concentrations of the celloidin or parlodion. The first concentration was a 2 per cent solution and each series increased 2 per cent until the final concentration was 12 per cent. The material on the agar was taken from the test-tube after fixing, cut into convenient pieces, and put through the above procedure. The pieces were kept in a tightly plugged bottle in an incubator at 45° C. and changes were made daily. After embedding, sections were cut to a thickness of 10 μ. These were then stained and mounted.

The best stain for this procedure was found to be iron-alum haematoxylin, using Heidenhain's haematoxylin, although Ehrlich's gave just as good results. A combination of Benda's fixing agent and iron-alum haematoxylin showed the reticulated network and the metachromatic material very well, whereas Hermann's fluid plus Heidenhain's iron-alum haematoxylin was best for nuclear structure. Methylene blue and Hermann's fluid brought out the vacuoles and volutin and the metachromatic material very clearly, as seen in pl. 23, figs. 15–18.

For morphological work, hanging-drop cultures were made of 2 per cent proteose peptone and 2 per cent bacto-peptone, as well as lactose broth cultures. Material was also placed in a drop of a 1 per cent solution of crystal violet (aqueous) in glycerine, the dye being added to the desired intensity. The preparation was allowed to stand from 15 to 30 minutes for a sufficient clearing. Aman's lactophenol was also applied, as was carbol fuchsin and very dilute solutions of methylene blue, eosine, and crystal violet. In addition, iodine potassium iodide (saturated solution) was used for studying the glycogen contents of the cells and chondriosomes, as advocated by Guilliermond. Osmic acid, platinic chloride, and iodine green were also used, as well as neutral red, but these materials and other methods of procedure will be explained later in the text.

Cytology

The cytology of the lower Ascomycetes, particularly the yeasts

and the yeast-like organisms, had been for a number of years a topic of discussion among the older cytologists. The question as to whether or not a nucleus was present was indeed a serious one, judging by the numerous and lengthy dissertations on the subject. As is customary, there were two sides to the argument. One group maintained that the cells, of the yeasts in this case, were made up of a mass of protoplasm and of nuclein, without a true nucleus, and that the nuclein is differentiated at times in the cytoplasm in the form of granules which assume a definite color on staining. The other group favored the presence of a definite nucleus.

The first reference to a nucleus in yeasts was perhaps made in 1844 by Nägeli, who decided that "a little nucleus of whitish mucus, lying on the membrane, regularly in each cell" (Wager, '98), was often found in the yeast cell. Whether Nägeli actually saw a nucleus in the fresh condition is rather doubtful, for an oil globule has often been mistaken for the nucleus even to-day, with our more advanced knowledge of cytology.

About five years later, in 1849, Schleiden, by treating cells with ether-alcohol or potash, was able to find a rounded structure with a clear cell wall which contained delicate granules either singly or in groups, and in addition, a large flat body, which he called a cytoblast.

Following this work, Brücke, in 1861, asserted that in living material, as well as dead cells treated with iodine and acetic acid, no definite nucleus could be seen and he reprimanded the former workers by saying that no one was justified in taking bodies of various sizes and numbers, such as often occur, for nuclei.

In 1879 Schmitz, using haematoxylin, was able to demonstrate a nucleus in the cytoplasm of the cell near the vacuole, while Strasburger, repeating this work in 1884 and 1889, using picric acid and haematoxylin, was able to confirm these observations, finding that the nucleus was not demonstrable in unstained material.

Krasser, in 1885, contradicted these findings by saying that granules existed in the yeast-cell, but no nucleus. His main argument was that there was no specific staining reaction for nuclei, and furthermore that the absence of a definite nucleus

in the yeast-cell was supported by the rapid growth of the organism. For this reason he believed that there was nuclein in the cell which was distributed through the protoplasm very much as is generally held to be the case with bacteria to-day.

Other workers who held the view that a nucleus, or at least a "corpuscle" as some called it, was present, the biochemical properties and physiological functions of which were analogous to those found in the cells of plants and of animals, were Hansen, Strasburger, Zacharias, Moeller, Buscalioni, Henneguy, Dangeard ('93), Janssens ('02), and Janssens and Leblanc ('98). In fact, the latter writers made the following statement, "*La cellule de la levure peut être considérée comme formé sur la type général de la cellule.* On y trouve en effet, un *noyau*, un *protoplasme* et *une membrane.*" To these workers the nucleus had a vacuolar appearance with a very differentiated structure, to others it was a homogeneous body. Wager ('98), in a detailed bit of work, described it as being a vacuole filled with chromatin granulations (perhaps somewhat as is shown in pl. 23, figs. 17–18) which had been taken for a nucleus by Janssens and Leblanc, and furthermore that a spherical and homogeneous body, considered by some as the nucleus, was always adjacent to the vacuole and could be compared with a nucleolus. He considered the vacuole plus the eccentric nucleolus as a primitive stage in the phylogeny of the development of the nucleus. This belief was also based on the observation that the small body and the vacuole divide simultaneously by budding.

Guilliermond, in 1902, demonstrated that a definite nucleus was present, and that the vacuole was independent of the nucleus, being filled with granulation products which at the present time he holds to be nuclear decomposition substances (nucleic acid derivatives with some unknown base). For the granules he retained the name "corpuscules métachromatiques," a term which had been applied previously and which is used even now.

Those who denied the presence of a nucleus but admitted the presence of a nuclein substance as described above were Raum, Roncali, Hieronymus, Macallum, and others.

In accordance with the views of the older writers, the author has found that in *Endomyces* as treated here, when first isolated

from the tissue of the host, no nucleus exists, but simply a distribution of the chromatin material or nuclein or metachromatic corpuscles throughout the cell, as may be seen in pl. 19, figs. 13–14, pl. 20B, pl. 21, figs. 1–3, and several others. This feature is a quite constant character of freshly isolated cells. However, when kept on agar or an artificial substrate, the cells not only change their form, passing through what may be termed the secondary stage, that is changing from a yeast-cell to a hyphal form, but also undergo a change in nucleoplasmic make-up. It has been found that even though the yeast-like cells retain the same morphological characteristics on agar media, their chromatin material or nuclein is converted into a definite nucleus. This was clearly shown to be the case in *E. dermatitidis* (Moore, '33). What the mechanism involved here might be, it is difficult to say, but we may conjecture that it is linked up with the adaptation to a changed environment and a different mode of development to which it must become accustomed, as shall be pointed out later.

With such views being held in the past, it was of course to be expected that the presence of any phenomena which might involve the nucleus, as mitosis, would also be in dispute. In yeasts the question of nuclear division has received considerable attention, particularly by Guilliermond, Dangeard, and several others, the former writing many and long papers on the subject, and it has been said by some present-day workers, and the following is a direct quotation from the papers of one of them, that "the ideas of Guilliermond . . . which gain weight by the mere bulk of his work on yeast, seem to meet with more favor."

The problem of the division of the nucleus in yeasts is perhaps more complex than would seem offhand. The same writers who held that a nucleus was present in the cell first described nuclear phenomena. Wager ('98) interpreted an amitosis in the yeast with perhaps evidence of chromosomes. Janssens and Leblanc ('98), working with *Saccharomyces cerevisiae* and *S. Ludwigii*, as well as *Schizosaccharomyces octosporus* and *S. Pombe*, described a form of mitosis which, in the light of other works, seemed very suspicious and they made the following statement, "On peut dire que tout ce qui diminue la vitalité d'une cellule tend à réduire la complication des phenomènes de division."

Dangeard ('93) found that the nucleus elongated and the nucleolus divided in two by an elongation to a thread-like process within the nuclear wall. Swellengrebel ('05) and later Fuhrmann ('06) ascribed a definite mitosis to the nucleus, the latter studying *Saccharomyces ellipsoideus* I. Hansen and presenting a typical karyokinetic sequence with the formation of four chromosomes. There were several authors who held to this view, and at present there are those who believe that definite mitotic phenomena are present. Kater ('27), using a smear technique with *S. cerviciae* [sic], demonstrated a mitosis, with the formation of about eight chromosomes and a definite spindle, instead of division by constriction. It should be pointed out here that smear methods as applied to yeasts and particularly as carried out by Kater have never yielded any results which might be considered reliable. The act of smearing, no matter how good or how careful the application of the fixative might be, usually allows for some action on the cell wall that obliterates the correct phenomena and substitutes artifacts, so that vacuolar constituents or secretion products of the cytoplasm have been misinterpreted.

On the other hand, Guilliermond ('17), in a summary of his work, found amitosis to occur where budding was present. This process was characterized by an elongation of the nucleus which quickly divided by the resorption of the thread-like portion that separated the two segments. Guilliermond also observed that it was impossible to see the nuclear phenomenon clearly due to the abundant products of secretion which covered the nucleus. The observations of Kohl, of Wager and Peniston ('10), and of Pénau confirmed Guilliermond's results, and even went so far as to actually claim definite amitosis.

Whether or not mitosis or amitosis actually occurs is still a matter of dispute, despite the evidence that either side may advance. There are several factors which must be considered in studying the process, or processes, in nuclear division. First, does the nucleolus have a single morphological characterization, such as exists in the higher forms, or does it constitute the total nucleoplasmic material, as some call it, which has an affinity for iron haematoxylin, for example, as in pl. 22, fig. 18? Second,

can the nucleus be seen so clearly that there is no mistaking the change that may take place during the actual process? The first question is difficult to answer because it has been found by several workers studying the chemistry of the cell, yeast or yeast-like or even that of the higher Ascomycetes, that certain materials take the same dye. Then, the linin network with the chromatin material or the chromomeres, as some call them, usually cause confusion. The second question is really serious, because we know that our most precise methods of technique are still too crude for the minute nuclear make-up of these lower Ascomycetes. Cell substances, or secretion products as Guilliermond calls them, usually take stains which are peculiar to the nucleus and mask any clear-cut pictures of nuclear phenomena, and nucleic acid substances, derivatives of excretion products of the nucleus, probably nucleophosphates and the like, are also substances that must be considered in detail. Besides, the size of the reactors in the process is too small for any precise determinations with the means at hand.

On the other hand, according to the theories of modern genetics there must be some mechanism whereby chromatic substance or material is distributed through the agency of mitosis, whereas the act of amitosis is simply a means of increasing the nuclear surface or spreading the nuclear material through a cell and is comparable to the fragmentation or lobulation of nuclei. The latter is not at all reproductive in this respect. However, there are many acts taking place in the daily life of a fungus, particularly in these lower groups, which are impossible to explain with our present knowledge of cytology or genetics, and theories have been built up only to be torn down and built up again. This does not mean that genetics is not standing on firm ground, but that much more must be learned about mycological phenomena before any generalizations can be made.

It is difficult to understand that the nucleus in the hyphae of yeast-like organisms, *E. capsulatus* or *E. dermatitidis*, divides by direct division, at least as far as can be made out from the nuclear appearance. The process is evidently very rapid, for an examination of a great many slides failed to show any condition other than that shown in pl. 22, figs. 3–5. In the

ascogenous hyphae, or perhaps the antheridium and ascogonium (pl. 22, figs. 6–9), the division is somewhat slower, and here an elongation of the nucleus and nucleolus may be seen with a deeper staining central portion indefinite as to character but perhaps analogous to chromosomes.

In a discussion of nuclear phenomena, the next thing to be considered is the mode of development or reproduction. Guilliermond ('05, '05a, '05b, '08, '09, '09b, '10, '10a, '10b, '11, '11a, '12, '13, '17, '19, '20), Dangeard ('93, '94, '94a, '94b, '97), Hansen ('04), and several others, in a long series of investigations, have described the sexuality of the lower yeasts and some of the yeast-like fungi. It is known, of course, that in the Ascomycetes, particularly the lower forms, there may be three forms of development, heterogamy, isogamy, and a reduction to parthenogenesis. In the first case, two gametes, usually of unequal size, a small one generally representing the antheridial cell and a larger one the ascogonium (this is not a hard and fast rule), send out one, or sometimes several, small tubes which copulate and fuse. The cell contents of the antheridium then pass into that of the ascogonium. The two nuclei, one from each gamete, fuse, and finally an ascus develops through the subsequent division of the fusion nucleus, the presumptive mother spore. In the case of isogamy in the yeasts, two morphologically alike gametes fuse in like manner. Finally in the series, there is a reduction to a condition in which a cell may suddenly produce spores without copulation. This condition has been found by Mangenot ('19) for *E. Lindneri* and by Guilliermond for several other species, as *E. fibuliger* and *Zygosaccharomyces Pastori*. It occurs also when an ascogonial cell sending out a tube or tubes fails to copulate and hence produces spores parthenogenetically.

In some species the above phenomena may be found separately, or two processes or even all three may be present in the same culture. This latter condition has been found to be true for *E. capsulatus*.

In addition to the parthenogenetic formation of asci and ascospores, there is a non-sexual reproductive structure which has not been given too much attention in the past. This is the conidium (pl. 23, fig. 10), which is a non-nucleated structure

filled with chromatin granules or the metachromatic corpuscles emphasized by Guilliermond. It occurs usually near a septum of the hypha, and measures approximately 5 μ in diameter. Structures of this sort may and usually do spring up from the hyphae when there is a lack of nutrient material, or when the hydrogen-ion concentration is fairly high (pl. 19, figs. 4, 7–8, 11–12). These may be pyriform (pl. 18, fig. 10) or round (pl. 18, fig. 7), sessile or on a short pedicel, with a thick wall (pl. 23, fig. 10) or a thin wall. There is a heavy reticulated network with the granules mentioned above usually occurring at the nodes of the threads. These particular structures break off easily and may serve as resting cells or chlamydospores which, when placed in favorable media, germinate, form nuclei by an accumulation perhaps of the chromatin material, and develop a normal growth. They represent probably a degeneration from an ascus, or, on the other hand, an advanced character of reproduction.

To get a clearer understanding of the life history of *E. capsulatus*, it would be advisable to begin with the organism as it is found in the parasitized host and explain the mechanism by which it and related fungi propagate themselves.

In the tissue the organism grows as a yeast 6–8 μ in diameter, and reproduces itself by budding. There is no nucleus present, at least it could not be demonstrated in tissue sections or in freshly isolated cells, but instead there is a distribution of nuclein material throughout the organism, much the same condition as exists in bacteria, Myxophyceae, or in the conidium shown here. When isolated and grown on an artificial substrate as on agar, the chromatin or nuclein material seems to become larger, and there is usually formed a nucleus and many large granules which may be presumptive nuclei. This latter condition is based on circumstantial evidence (pl. 21, figs. 4–9; pl. 20B). The cells then pass through this stage, the yeast-cells, to stage two which consists of large irregular cells, attaining a condition as seen in pl. 19, figs. 14–15, 20–25, and have been clearly shown for *E. dermatitidis*. At this stage the chromatin material is spread throughout the cell, but the reticulated network seen in the later stages is not very clearly established. However, it seems to be rapidly developing and nuclei are clearly distinct. The stage

following is probably the most complex of all, inasmuch as there is a diversity in morphology and cytology, changes due probably to hydrogen-ion concentration and temperature and several other factors among which the changed habitat is outstanding. In this third and final stage, we find that sexuality has developed and asci with ascospores are produced. To follow the life cycle on an artificial substrate, it would then seem best to follow the development from the germinating ascospore. In doing this, it is necessary to consider first normal growth on some favorable medium, and second to correlate the nuclear changes in material fixed in hanging-drop preparations (Van Tieghem cells) and stained, with the nuclear phenomenon found in the material fixed and stained as outlined in the technique. It was noticed that from three to fifteen days were necessary for the life cycle of the fungus to be completed, depending on the broth used in the hanging-drop preparation.[2] It was found that proteose peptone broth gave good growth as did lactose broth, but that bacto-peptone plus bacto-beef gave quickest development of asci, from three to five days usually. The material was fixed by the addition of two or three drops of a fixative described previously, and after a period of from six to eight hours, or over night, the fluid was drawn off with filter-paper and the material was carefully washed with distilled water and then mounted in lacto-phenol plus crystal violet, or stained with iron haematoxylin. By keeping several of these hanging-drop cultures growing, it was possible to obtain different stages of growth, even in one preparation. The method was of course not entirely accurate cytologically, but it served the purpose as demonstrated here.

The single spore (2–2½ μ) is found to be made up of densely staining granular material, but the granules are so small that it is difficult to discern them. On germinating, a tube is sent out or the spore simply elongates, taking with it the chromatin material (pl. 22, figs. 1–2). When somewhat older, the developing hyphae produce nuclei which divide rapidly as pointed

[2] Several solutions were used: lactose broth (product of Digestive Ferments Co.), pH 6.8; 2 per cent bacto-peptone broth, adjusted to pH 7.0; 2 per cent proteose peptone broth, pH 7.0; bacto-peptone plus 6 per cent glycerine; meat extract broth, pH 7.1; 2 per cent bacto-peptone plus 5 per cent bacto-beef (dehydrated), pH 7.2.

out previously, are distributed in pairs very close together, and then separate (figs. 3–4). At this time the reticulum is well developed and the chromatin granules, or basophilic grains, as Guilliermond calls them and which he believes probably represent albuminoid bodies playing the rôle of products of nutrition in the form of perhaps zymogen or reserve material, are very evident and take a deep stain with haematoxylin. At the same time, the wall increases in thickness and becomes fairly evident as in fig. 4. The number of nuclei in the hyphae varies, as many as thirty-seven having been counted on one hypha, but this was apparently rare for the amount usually varied between seven and fifteen with nine the most common, as in fig. 4. With the elongation of the new hypha, septa are laid down (fig. 5) and the formation of conidia as described previously (pl. 22, fig. 5; pl. 19, fig. 4) may be and usually is associated with this act. Following this, the hyphae, varying from 1½ to 4 μ on different media, may grow out to form branches or reproductive structures, usually lateral or terminal. However, on acid media or on media where there is a definite lack of some suitable protein or protein product in the form of peptone, peptides, or amino acids, there is an excessive formation of chlamydospores, either terminal (hypnospores), 4½–6 x 9–12 μ, or lateral, 5 x 7 μ, or intercalary, approximately 5 μ in diameter, and conidia (pl. 18, fig. 7; pl. 19, figs. 7, 11–12). With the apparent development of a hypha, there is an accompanying formation of sexual cells. This process may be slow or rapid, depending on conditions. On glycerine agar it seemed to be the most rapid, these particular organs being produced in three to five days. The antheridial or male cell contains two or three nuclei as a rule (pl. 22, figs. 6–8), but cases of four have also been seen. The ascogonium or female cell usually contains three to four nuclei (figs. 9–11), but five (fig. 12) have also been noticed.

When two sexual cells are ready for copulation, the antheridium bends over to meet the ascogonium as seen in pl. 22, figs. 10–11. The two gametes may be on the same hypha or on separate hyphae. Both may be terminal (figs. 13–17), or both lateral (fig. 16), or one may be lateral and the other terminal (fig. 12), or there may even be variations of these. Nevertheless it is

quite apparent that the male cell goes to meet the female cell. With the approach of the copulation branches, there is an action which may be termed tropistic and which may be explained, if analogies are acceptable, by a hormone action or physical stimulus as is found in zoological specimens, which calls forth the production of a beak, the copulating tube, as has been found in the lower yeasts. These two beaks meet and fuse. In the meantime, one of the nuclei proceeds to the tip of the beak. There is probably no specific nucleus involved in this act, because as far as can be made out all the nuclei are the same morphologically, and any nucleus that happens to be nearest the beak becomes sensitized to the fertilization reaction. With the progression of the fertilization nucleus, there is an accompanying retrogression of the remaining nuclei. Whether these disintegrate or not has not been definitely determined. In several cases where fertilization had taken place, it was noticed that the antheridial cell and basal portion of the ascogonium merely existed very much like functionless stamens in a flowering plant, which in the course of time disintegrated or perhaps degenerated.

The next step in the cycle involves the fusion of the nuclei, or syngamy perhaps. The walls between the two tubes are dissolved or diffuse and the two nuclei copulate or fuse in the tube (pl. 22, figs. 13–16). This is rather contradictory to Dangeard's ('94, '94a, '94b, '97) observations that the two nuclei fused in the ascus, and Harper's ('96, '97, '99, '00), that in the higher Ascomycetes the fusion occurred in the archicarp after the entrance of the contents of the antheridium. The fusion nucleus is then transferred or migrates (whatever the process may be it is analogous to that by which the nuclei copulate) into the ascogonium which is now known as the archicarp (pl. 22, fig. 17). The fusion tube dissolves in some cases or breaks away in others, with the broken portion being either resorbed by the cells or degenerating. At any rate, the archicarp repairs its walls by the resorption of the cellular contents, and the large fusion nucleus is ready for division (fig. 18).

The nucleus divides by a process of amitosis as previously mentioned. Although difficult to interpret theoretically, it presents no clear-cut chromosomal formation. With the first

division, there is apparently the formation of a wall (pl. 22, fig. 19). The daughter nuclei go through a synchronous division (fig. 20) to form four nuclei. In several of the yeasts and in *E. Magnusii*, the process halts here and each nucleus develops into a spore, thus four spores. Here, however, the four nuclei undergo a synchronous division, the second for the ascus, and eight nuclei are produced, which number is a mean for the species of this genus. In other species there may be twelve or sixteen nuclei.

In the higher Ascomycetes the problem of ascus formation is rather confusing, and the reader is referred to the work of Atkinson ('15), Bagchee ('25), Blackman and Fraser ('05), Brooks ('10), Brown ('10, '11), Carruthers ('11), Guilliermond, Mangenot and Plantefol ('33), Faull ('05), Fraser and Brooks ('09), Fraser and Welsford ('08), Gaümann and Dodge ('28), Harper ('96, '97, '99, '00), and Maire ('04, '05), to obtain full information and various opinions on the subject.

After the formation of the nuclei and the abjunction of the future ascus (8–14 μ in diameter) from the basal portion of the ascogonium, now a basal cell as seen in pl. 22, figs. 21–22, the development of the spores begins. Associated with this phenomenon are the secretion products which, as has been pointed out previously by Guilliermond, nourish the spores and produce the spore wall.

It should be pointed out here that, in addition to this heterogamous form of copulation, isogamy and finally parthenogenesis may also be present. The latter is quite apparent and has been found time and time again. The process may begin by the jutting out of what is apparently a conidium. This in turn develops and a nucleus becomes visible. The nucleus divides as noted in the above reaction and an ascus with eight ascospores forms (pl. 23, figs. 1–9). Also a terminal cell often produces a pyriform ascus parthenogenetically as seen in fig. 11. In all cases, the spores serve the same function and are equally as capable of producing new hyphae. The sporogenous plasma of the ascus, part of the cytoplasm, contributes greatly to the formation of the mature spore.

The life cycle of *E. capsulatus* on an artificial substrate might be graphically illustrated as in diagram 1.

Cellular contents.—The remainder of the ascus constitutes the epiplasm which is made up of reserve products in the form of lipoids, glycogen, the metachromatic granules of Guilliermond or nuclear decomposition products as volutin, oil globules, and nucleic acid substances and probably other protein derivatives and carbohydrates. All of these materials have been shown to be utilized by the spores in their growth. Several authors also present evidence that during the maturation process of the spores,

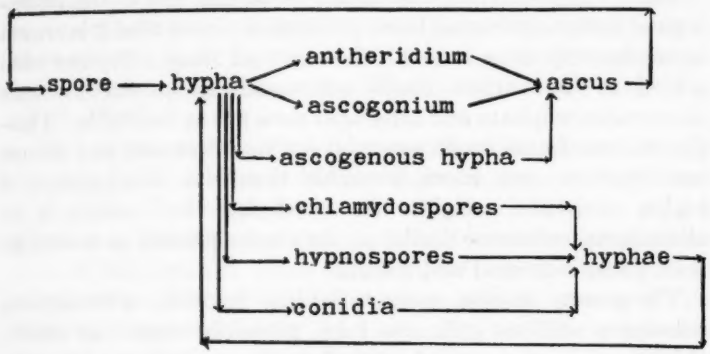


Diagram 1.

the substances constituting the epiplasm are broken down, a part being absorbed and clearly demonstrable in the granular contents at maturity, and a part reserved for their germination.

Volutin.—The cellular contents will be considered in the order studied. The first is what has been variously called "Neisser's granules" found by Neisser in bacteria; "sporogenous grains" found by Ernst; "metachromatic corpuscles" of Babès, who found them in the diphtheria bacillus and so named them because of their metachromatic action; "red grains" of Bütschli, so named because they took on a red coloration with many of the stains; and finally, one of the most common of all, volutin, as named by Meyer.

In the fresh state these granules stain blue with methylene blue (pl. 23, figs. 16–18), and when fixed their color is red to violet. They are small at first, and then enlarge to assume various forms and sizes. They may have a heavy staining outer portion and

a paler center, probably due to their refractive powers. They are seen quite often in vacuoles as represented here and assume fantastic features. They appear in the cell along the reticulated network, at the nodes, and stain heavily there. Volutin is very common in the younger cells, but not so abundant in the older hyphae. The substance may also be stained *in vivo* with neutral red. Wager and Peniston ('10) found Gram's aniline violet to be a useful stain for volutin.

Zikes ('22), studying the nature of volutin and of the physiological factors concerned in its production, found that it is present in moderately large amounts in nearly all fungi. Peptone when added to the culture media stimulated its production while ammonium sulphate and asparagin were not so favorable. Phosphorus was found to be essential for its formation, and glucose and fructose were more favorable than the carbohydrates of higher molecular weight. He concluded that volutin is an albuminous substance similar to the nucleo protein, as it contains both phosphoric acid and nuclein.

The general opinion seems to be that volutin is a nucleic acid substance with an unknown base, probably organic in nature, that it exists as a colloid, and that, due to fixatives, it is precipitated in the vacuoles. It is supposedly a secretion from the nucleus and is used in nourishing the spores.

Glycogen.—Another substance which supposedly acts as a reserve material and is used up for the maturation of the spores in the ascus is glycogen. This theory has been substantiated by the fact that glycogen is used up in the cell, particularly in maturing asci, as evidenced by its slow disappearance. Kohl ('07) considered glycogen not as a reserve material, but as a regulator for the intake of sugar in the cell, in that it is the substance formed, not being able to diffuse out from the membrane. He found it to be lacking in spores, and since reserve materials should be present, he presented this as evidence that glycogen is not a reserve material. Guilliermond, however, claims to have demonstrated that glycogen is absorbed by the ascospores at the time it is found to disappear from the epiplasm.

Glycogen is found in the organism practically from the beginning of growth, first as small droplets perhaps, but later in

fairly large masses (pl. 23, figs. 19–30). It has been estimated as constituting 32 per cent of the dry weight of yeast cells. It is associated with the nutritive condition by some workers and found to be almost entirely used up or greatly accumulated according to the condition of nutrition and growth. According to some workers it appears somewhat in the form of a vacuole and it has thus been called a glycogen vacuole. It is not a true vacuole, but perhaps a colloidal substance, as volutin is considered to be, for it takes a stain much as the latter does. It may be stained *in vivo* with neutral red (figs. 26, 29–30), in which case it is not so clear, at least in *E. capsulatus*, as with a saturated solution of iodine potassium iodide, a modification of Gram's stain (figs. 19–25, 27–28).

This substance is a glucoside, a polysaccharide which, like starch, is made up of n molecules of glucose to have the formula $(C_6H_{10}O_5)_n$. It is often compared with starch since it requires phosphorus for its formation. In fact, it has often been referred to as an amylopectin, which makes up the superficial part of the starch grain. By hydrolysis, glycogen is broken down to dextrin, then maltose, and finally glucose. On heating a saturated solution of glycogen stained with iodine potassium iodide it becomes pale but regains its intensity on cooling. It is insoluble in alcohol.

Vacuoles.—The question of vacuoles in fungi has been given considerable attention in the past, and the author does not intend to enter into a discussion of them. However, in view of the many theories advanced as to their character and presence, mention of them in *E. capsulatus* would not be out of place. They are easily demonstrable with many dyes and present varied characteristics. With methylene blue, they are easily made visible (pl. 23, figs. 15, 17–18). They appear to be formed by the reticulum and to take the stain. They may be demonstrated fairly clearly with iodine green (fig. 14), and the standard dye seems to be neutral red, in which case small crystalloid bodies, often described as "dancing bodies," probably Brownian movement, may be seen (figs. 26, 28). Haematoxylin also brings them out distinctly by staining the surrounding network (figs. 1–2). What the function of vacuoles may be is difficult

to understand. It is known that they are colloidal solutions of cytoplasmic materials, formed by the absorption of water, that they may enlarge or disappear in a cell, and Guilliermond, Mangenot and Plantefol, say this of them, "Les vacuoles représentent la phase aqueuse du cytoplasme et jouent certainement le rôle important en réglant la teneur en eau de la cellule."

Chondriosomes.—The bodies designated as mitochondria or chondriosomes have been demonstrated in animal and plant tissue. What their particular function may be is not definitely known. In plants they have been associated with the elaboration of chlorophyll and starch through the agency of the chloroplasts. In animals they are supposedly associated with the elaboration of secretions, but no definite evidence has been produced along this line. They have been demonstrated in a number of lower plants, lower fungi and the higher Ascomycetes, and in fact, in practically all of the groups, as well as in animal cells. For further information the reader is referred to Guilliermond ('11b), who has traced the development of these particular bodies in many organisms.

Chondriosomes (Cowdry, '17) have been shown in both animal and plant cells to be almost completely soluble in alcohol, ether, chloroform, and dilute acetic acid (organic solvents) and to be insoluble by chromization and treatment with formalin, at least in most cases. In the animal cell they do not stain with Sudan III or IV and are sometimes blackened with osmic acid. In plants, the whole cell blackens with osmic acid so readily that it is impossible to know just to what extent the chondriosomes themselves are affected.

In studying *E. capsulatus*, the author made use of the knowledge that chondriosomes are very clearly demonstrated by the addition of iodine potassium iodide to a preparation of an organism. They are fairly evident as lightly staining rod-like and short bodies (light yellow with iodine), but one must pay particular attention to distinguish them (pl. 23, figs. 19–25). There are short and rod-like bodies in the terminal hypnospores and some long forms too, as well as small point-like reflective granules. The rods are longer in the hyphae (figs. 23–24). Chondriosomes are scattered throughout the asci as fine, small, rounded bodies.

It can thus be stated that by the iodine potassium iodide method, bodies comparable to those described as chondriosomes for other fungi are demonstrable.

Fat, lipoidal substances.—In addition to the many substances mentioned, there are also fats, lipoidal substances, and other materials called reserve materials, secretion products, and also excretion products. These materials are found in varying amounts in many of the specialized portions of the organism, being abundant in the asci and chlamydospores and in very small amounts in young hyphae and younger elements. They can be demonstrated comparatively easily in spores and in yeasts particularly. The nature of many of these substances has not been determined as yet, but it is generally known that fatty acids, glycerides and sterides, glycerol, phospholipides and phosphoaminolipides (complex lipides) may be present in certain amounts.

Several agents were used for the study of these materials, each giving some degree of difference in distinguishing them. A 2 per cent osmic acid solution which reduces fats and gives them a black coloration was the first tried. When applied to the living mycelium, the fats and lipoidal substances appear as small refractile bodies or droplets but in some cases they are very much larger (pl. 23, figs. 31–36). They are seen rather abundantly in old hyphae (fig. 36) and in very small amounts in young hyphal tips (fig. 35). With platinic chloride (5 per cent solution) they appear as blackened granules much the same as with osmic acid (figs. 37–38). Iodine potassium iodide as applied for glycogen and chondriosomes shows lipoidal substances, as oil droplets, equally as well. These are very small, highly refractile, and hyaline, and to be seen require careful focusing and adjustment of the microscope (figs. 19–20, 23–25). Neutral red has also been used, but not much attention was paid to the reaction outside of what has already been mentioned with regard to glycogen and the vacuoles. Iodine green also has an affinity for lipoidal substances, but not enough attention was given to it to prove its value here.

Cultural Characteristics

In studying the cultural characteristics of *E. capsulatus*, all the media available at the time were utilized. This was considered essential, inasmuch as it has become an unfortunate custom with some mycologists to name as new species organisms which show a physiological variation on a different medium. Since the fungus had shown on previous occasions of culturing that it favored protein substrates, a variety of protein materials was chosen. In addition, regular routine media were used, some with much carbohydrate and some with very little. For some years the question of hydrogen-ion concentration has become an important factor in work of this sort, and media with a wide range of pH were chosen within which the possibilities of growth had at one time or another been emphasized. All cultures were grown at a temperature of approximately 25° C.

The following media arranged in the order of their decreasing hydrogen-ion concentrations were used:

Raulin's Solution Agar (*Raulin's solution plus 1.5 per cent agar, pH 4.1*).—(pl. 18, fig. 7; pl. 19, figs. 8, 10–12). Growth poor, being present only around inoculum after 18 days and having a diameter of only 0.7 cm. and 1.6 cm. at end of 30 days. Color white. Hyphae grown heaped up from center in a loose fashion. Microscopically, abundance of conidia, pedicelled or sessile, 5 μ in diameter; endo-chlamydospores 5–7 μ in diameter; terminal hypnospores 4–6 x 8–11 μ; hyphae 2 μ in diameter; racquet mycelium in small amount, not very noticeable; asci 9–10 μ in diameter.

Richards' Solution Agar (*Richards' solution plus 1.5 per cent agar, pH 4.3*).—(pl. 19, figs. 5–7, 9). Growth slow, very sparse and cottony, with long and narrow hyphae 2 μ in diameter, projecting loosely and irregularly from the edge of the colony which attained a diameter of approximately 1.8 cm. at end of 18 days and 3.5 cm. at end of 30 days. Round conidia very numerous, approximately 4 μ in diameter; pyriform conidia sessile or on short stalks, several, 3½ x 7 μ in diameter; round, thick-walled chlamydospores on short pedicels, 5 μ in diameter; asci few, 7–9 μ in diameter; endo-chlamydospores approximately 6 μ in diameter.

Czapek's Agar (*pH 4.4*).—Growth of very loose and sparse mycelium which spreads over the surface of the agar with thin hyphae. Macroscopically the culture is barely visible except by reflected light. Colony approximately 3 cm. in diameter at end of 18 days and 6.7 cm. at end of 30 days. Color white. Hyphae 2½–3½ μ in diameter; racquet mycelium present, 6–7 μ in diameter at swollen portion and 2½–3 μ at thin portion; terminal hypnospores 7 x 12 μ approximately; conidia many, 5 μ in diameter; round chlamydospores 6 μ in diameter; lateral chlamydospores 6 x 11 μ; asci several, 10 μ in diameter.

Wort Agar (*Product of Digestive Ferments Co., pH 4.6*).—(pl. 18, figs. 6, 8–10, 15). Growth at first slow, none on several of the cultures, in general thin and loose. One of the cultures showed good growth, attaining a diameter of 3.2 cm. at end of 18 days and 5 cm. at end of 30 days. This was unusual and may be ascribed to too great an inoculation and to a dissemination of spores as a result of shaking. Color white. Terminal hypnospores many, 7 x 12 μ; hyphae 3½–4 μ in diameter; chlamydospores 6½–7 μ in diameter; racquet mycelium abundant; conidia numerous, 5–6 μ in diameter; asci 10–11 μ in diameter.

Malt Extract Agar (*pH 5.1*).—(pl. 18, figs. 1–3). Growth not so abundant, colony 1.6 cm. at end of 30 days. Color slightly brown, due to the malt extract. Mycelium of numerous swelled cells; hyphae 3–4 μ in diameter; terminal hypnospores 6 x 16 μ; hyphal swellings or chlamydospores 9 x 12 μ; numerous conidia, pyriform and round, the round being approximately 5 μ in diameter; asci very few, 10 μ in diameter.

Malt Extract Broth (*The above minus the agar*).—Very little growth at end of 30 days. Characteristics same as above.

Maltose Agar (*pH 5.4*).—Growth slow. Colony coremium-like, with a diameter of 1.5 cm. at end of 30 days. Hyphae short, 3–4 μ in diameter. Many round chlamydospores 5 μ in diameter; terminal hypnospores numerous, 4–6 x 9–11 μ; asci few, 8–10 μ in diameter.

Sabouraud's Broth (*Sabouraud's dextrose agar minus the agar, pH 5.5*).—(pl. 21, fig. 21). Culture consists of submerged colonies of mycelium varying from ½ to 3 cm. in diameter at end of 18 days, with a great mass at the end of 30 days as a result of the

coalescence of all the colonies. The large flakes are grey in color when moist, but with white mycelium on the surface, fairly dry. Submerged hyphae 2½ μ in diameter with none or very few morphological characteristics. Aerial or dry mycelium, however, similar to that on the agar culture of the same medium.

Sabouraud's Agar (*pH 5.6*).—(pl. 16, figs. 12–14; pl. 19, figs. 13–25; pl. 21, figs. 14, 18, 28). Thick cream-colored growth with a diameter of 4.5 cm. in 18 days and 7 cm. in 30 days. Hyphae 2–3½ μ in diameter, in needle-like projections from the surface of the mycelium which appears very cottony. Color white. Older cultures show a felt-like matting with a tendency towards ridge formation, a condition found to a certain extent in *Microsporon Audouini* Ota and Langeron. Racquet mycelium present with swollen portions 4–6 μ and narrow section 3 μ; many lateral chlamydospores, 5–7 x 10–12 μ; terminal hypnospores 5 x 11 μ; and many conidia, pyriform or round, approximately 5 μ in diameter; numerous asci, 10–12 μ in diameter, with 8 ascospores 2–2½ μ in diameter.

Oat-Meal Agar (*Decoction of oat-meal plus dextrose and agar, pH 5.9*).—Growth diffuse and slow, 5 cm. in diameter after 30 days. Culture loose and cottony. Hyphae 2–2½ μ in diameter; numerous thick-walled, round resting cells or chlamydospores, 4–6 μ in diameter; terminal hypnospores 4 x 7 μ; conidia numerous, 4 μ in diameter; asci several, approximately 8 μ in diameter.

Corn-Meal Agar (*Product of Digestive Ferments Co., pH 6.0*).—(pl. 16, figs. 8–9; pl. 21, fig. 24). Growth very loose and thin, with the hyphae projecting from the colony, appearing as threads of silk. Colony barely visible except from a lateral view. Hyphae long and thin, 2–2½ μ in diameter, with colony attaining a diameter of 5 cm. at end of 21 days and 6 cm. at end of 30 days; chlamydospores 6 μ in diameter; conidia many, 4½–5 μ in diameter; terminal hypnospores 5 x 7 μ; racquet mycelium present but reduced in size, 3–4 μ at swollen portion; asci several, 7–8 μ in diameter.

Potato-Dextrose Agar (*Decoction of potatoes plus dextrose and agar, pH 6.2*).—(pl. 21, fig. 22). Colony 3.6 cm. in diameter at end of 30 days, edge smooth and round, no striations or other cultural changes as ridges. Growth thick and cottony at in-

oculum, with a thin periphery about 2–3 mm. in width. Color white. Hyphae 2–2½ μ in diameter and long; conidia numerous, 5 μ in diameter; terminal hypnospores few, 4 x 7 μ; chlamydospores rare; asci few, 8–10 μ in diameter.

Two Per Cent Aqueous Bacto-Peptone (Hanging-drop culture, pH 6.2).—(pl. 21, figs. 1–8, 25, 32). Mass of thick-walled hyphae ramifying and branching, 2–2½ μ in diameter; racquet mycelium not evident; numerous chlamydospores, 4 μ in diameter; asci 4–6 μ in diameter; few terminal hypnospores, 3.5–5 x 6–7 μ.

Two Per Cent Aqueous Bacto-Peptone Plus Five Per Cent Meat Extract (Hanging-drop culture, pH 6.2).—(pl. 19, figs. 1–4; pl. 21, figs. 9–13). Growth profuse, covering the drop in 4 days. Hyphae 2–2½ μ in diameter, with an abundance of racquet mycelium intertwining and branching; thick-walled cells, chlamydospores in abundance, as well as asci 10 μ in diameter.

Two Per Cent Proteose Peptone Plus Six Per Cent Glycerine (Hanging-drop culture, pH 6.2).—(pl. 16, fig. 7; pl. 17; pl. 21, figs. 15, 30). Growth similar to that on bacto-peptone broth, but with an abundance of chlamydospores and racquet mycelium.

Lactose Broth (Product of Digestive Ferments Co., pH 6.8).—(pl. 21, fig. 17). Growth of submerged large flakes of colorless mycelium measuring approximately 2 cm. in diameter at end of 18 days. These later coalesced or intertwined into a mat which grew up the sides of the flask to form a white mycelium. Submerged hyphae approximately 3 μ in diameter, branching, intertwining, with cross-walls. Swellings, chlamydospores, terminal hypnospores, and asci few in number and reduced in size as compared with those on agar. The aerial mycelium above the surface of the broth showed an increased number of characteristics which simulated those found on the agar.

Lactose Agar (The above medium plus 1.5 per cent agar, pH, 6.8).—(pl. 18, fig. 11; pl. 19, fig. 20). Growth rapid, thick and cottony, colony attaining a diameter of 3.6 cm. at end of 18 days and 6.2 cm. at end of 30 days. Culture showed 4 ridges radiating from a thick inoculum to a thick cottony circular periphery surrounded by a thin growing rim of hyphae. Culture similar to that on nutrient agar, macroscopically. Hyphae 2½–3½ μ in diameter; conidia many, 4½–5 μ in diameter; terminal hypno-

spores 4 x 7½ μ; racquet mycelium present in abundance; many chlamydospores, varying from 6 μ in diameter (round) to 5 x 8 μ (pyriform); asci many, 8–9 μ in diameter.

Nutrient Agar (*Product of Digestive Ferments Co., pH 6.8*).—(pl. 21, fig. 19). Growth good, with the colony having a diameter of 3.5 cm. at the end of 20 days and 7.0 cm. after 30 days. Growth loose and cottony, showing concentric circles, evidently due to a periodical formation of asci and the liberation of the spores which germinated. Single spore colonies 2 cm. in diameter after 15 days. Colony flat with age, and white. Hyphae 2–3 μ in diameter; conidia many, sessile or pedicellate, 5 μ in diameter; chlamydospores several, 6 μ in diameter when intercalary, 5 x 7 μ when lateral, 6 x 11 μ when terminal (hypnospores); racquet mycelium present, 5 x 3 μ.

Nutrient Broth (*Meat extract, pH 6.8*).—(pl. 21, fig. 31). Culture similar to that on lactose broth.

Uschinsky's Protein-free Medium (*pH 6.8*).—No growth.

Beef Extract Agar (*Liebig's extract of beef, pH 7.0*).—(pl. 16, fig. 15). Growth similar to that on lactose agar. Radiating ridges present.

Eosine-Methylene-Blue Agar (*Product of Digestive Ferments Co., pH 7.0*).—(pl. 21, fig. 27). Growth good, attaining a diameter of 5 cm. after 26 days. Color pink in younger portion of culture (periphery), darker pink to blue towards the inoculum, this being due to an absorption of the dyes by the mycelium. Growth ceased and colony became flat. Hyphae in growing culture 2–3½ μ in diameter, with morphological characteristics similar to those on Sabouraud's agar.

Glycerine Agar (*Nutrient agar plus 6 per cent glycerine, pH 7.1*).—(pl. 16, figs. 1–3, 11; pl. 21, figs. 16, 23, 26, 29). The colony on this medium grew as a cerebriform, very thick, creamy culture, having a diameter of 6 cm. at the end of 30 days. Medium best for study of organism because of its nutrient constituents, presence of abundant protein and carbohydrate, and an abundance of endo-chlamydospores, 5½–6 x 11 μ; intercalary chlamydospores 4–5 x 5–7 μ; terminal hypnospores 4–6 x 9–12 μ; hyphae 2½–4 μ in diameter; round chlamydospores, either terminal or lateral, approximately 6 μ in diameter; racquet mycelium abundant; asci numerous, 10–14 μ in diameter.

Blood Agar (*pH 7.2*).—(pl. 16, figs. 4–6). Growth similar to that on nutrient agar.

Chocolate Agar (*Blood agar heated to about 75° C. until the blood became chocolate colored, pH 7.2*).—Growth similar to the above.

Serum Agar (*Beef extract agar plus 10 per cent dehydrated blood serum, pH 7.2*).—(pl. 18, figs. 4–5, 12–14, 16). Growth moist, showing a diameter of 1.4 cm. at end of 10 days and 3.5 cm. at end of 30 days. Colony flat and even. Many yeast-like cells seen; hyphae few and several large cells. Center of colony cerebriform. With age the culture shows prickly forms of mycelium (dry) which covers the greater part of the culture. This evidently is a reversion to the yeast form, to a certain extent.

Calcium Carbonate Agar (*pH 7.4*).—(pl. 16, fig. 10). Growth slow at first, then rapid, with a diameter of 7.4 cm. in 30 days. Colony compact, with a cerebriform central portion, and a loose, cottony outer zone. Color white. Hyphae 2–2½ μ in diameter; conidia numerous, 4½–5 μ in diameter, being spread throughout the entire culture, mostly round, several pyriform on short peduncles, others sessile; round chlamydospores many, 6 μ in diameter, and terminal hypnospores 4–5 x 7–9 μ; racquet mycelium not very evident; asci many, approximately 8 μ in diameter.

Endo's Agar (*Product of Digestive Ferments Co., pH 7.5*).—Growth slow, not quite so rapid as that on eosine-methylene-blue agar. Colony white at first, then, due to an absorption of the dye from the agar, pink to red, growing in coremium-like masses of straight hyphae with very few or no conidia. Hyphae short and thick-walled, 3½–4 μ in diameter. Few conidia in culture, 6 μ in diameter; hypnospores very few, as well as round chlamydospores; asci several, 10 μ in diameter.

Litmus Milk.—Milk was heated in a flask for fifteen minutes in steam, then set away over night in the ice-chest to allow the cream to rise. The cream was then siphoned off and the milk diluted in the ratio of 1 part milk to 4 parts water, with enough litmus as an indicator. Tubes of this solution were sterilized by steam, then inoculated, and kept at 25° C. No growth resulted after 20 days.

Reaction to Temperature.—In studying an organism, it is necessary, especially if quantitative results are desired, to determine conditions under which optimum growth can be obtained. One factor which enters into such considerations with fungi is temperature. It is known that various organisms grow best at certain temperatures and when placed in other conditions, growth will either be inhibited or retarded. With this point in mind, it was decided to grow a number of cultures on the same medium and same pH at various temperatures. As a medium, Liebig's beef extract agar at pH 7.2, which on previous occasions had shown a qualitatively and quantitatively good growth, was employed.

E. capsulatus, when first isolated from the lesions in its yeast-like form, showed very good growth at body temperature, 37.5° C. After having been kept in culture in its filamentous form for about two years, the optimum temperature was shown to be approximately 30° C. Continued growth on an artificial medium again reduced its optimum temperature, to approximately 25° C., as may be seen in table 1, or figs. 1–2.

Several cultures in petri dishes were grown at the various temperatures indicated in table 1. The experiment was performed at three different times, and due to the fact that daily measurements required handling of the cultures, several of the plates showed contamination. A procedure such as this usually brings in some foreign spores, especially in a large laboratory where many open cultures may be found.

The plates of the first two temperatures, −0.7° C. and 8.0° C., were kept in a Kelvinator, the former in the freezing unit where a fairly constant temperature was maintained, and the latter in a compartment some distance from the cooling device. The third set of plates, 16.0° C., was kept in a glass jar with running water around it. The temperature fluctuated somewhat, but not enough to affect the experimental data seriously. The plates of the other temperatures were kept in regulated incubators.

All the plates were inoculated with a loopful of a suspension mashed up with a sterile needle and contained 5 cc. of a beef extract broth solution of pH 7.2. In some cases the inoculum proved greater than others.

TABLE I

MEAN DIAMETERS IN CENTIMETERS OF COLONIES GROWN AT VARIOUS TEMPERATURES

Beef extract agar (Liebig's extract of beef) pH 7.2

Days after inoculation	Temperature in degrees Centigrade							
	−0.7	8.0	16.0	21.0	25.0	31.0	37.0	40.0
1	0.4	0.3	0.3	0.3	0.4	0.4	0.4	0.3
2	0*	0	0.3	0.4	0.5	0.4	0.4	0
3	0	0	0.3	0.4	0.8	0.7	0.6	0
4	0	0	0.3	0.5	1.0	0.9	0.7	0
5	0	0	0.4	0.7	1.3	1.1	0.9	0
6	0	0	0.6	0.9	1.6	1.3	1.1	0
7	0	0	0.8	1.1	2.0	1.6	1.3	0
8	0	0	0.9	1.4	2.2	1.8	1.4	0
9	0	0	1.1	1.7	2.5	2.0	1.5	0
10	0	0	1.3	1.9	2.7	2.2	1.5	0
11	0	0	1.4	2.2	3.0	2.5	0	0
12	0	0	1.6	2.4	3.3	2.7	0	0
13	0	0	1.8	2.6	3.5	2.9	0	0
14	0	0	2.0	2.8	3.8	3.2	0	0
15	0	0	2.1	3.0	4.1	3.4	0	0
16	0	0	2.3	3.2	4.4	3.6	0	0
17	0	0	2.5	3.4	4.7	3.9	0	0
18	0	0	2.7	3.7	5.0	4.1	0	0
19	0	0	2.9	4.0	5.2	4.4	0	0
20	0	0	3.1	4.2	5.5	4.7	0	0
21	0	0.3	3.3	4.5	5.7	4.9	0	0
22	0	0.3	3.5	4.8	5.9	5.1	0	0
23	0	0.4	3.6	5.0	6.2	5.3	0	0
24	0	0.5	3.7	5.2	6.5	5.5	0	0
25	0	0.5	3.8	5.5	6.8	5.7	0	0
26	0	0.6	3.9	5.6	7.1	5.8	0	0
27	0	0.8	3.9	5.7	7.4	5.9	0	0
28	0	0.9	3.9	6.0	7.7	6.0	0	0
29	0	1.0	3.9	6.2	7.9	6.1	0	0
30	0	1.1	3.9	6.5	8.2	6.2	0	0
31	0	1.2	3.9	6.7	8.3	6.2	0	0

*0 indicates no growth

Readings were made daily for 31 days, at approximately the same hour. The mean results of a representative series are given in table I. Three plates were kept in the final table as representive of the experiment although several additional plates were used.

An analysis of the results of this experiment shows that no growth takes place at −0.7° C. and at 40.0° C. At 8.0° C. growth does not begin until the twenty-third day and then proceeds slowly, with a maximum diameter of 1.2 cm. which would represent a total growth of 0.9 cm. At 16.0° C. the culture does not show growth until the fourth day, and grows slowly but faster than that at 8.0° C., to show a final diameter of 3.9 cm. or 3.6 cm. actual growth. At 21.0° C. there is a final diameter of 6.7 cm., with a total growth of 6.4 cm. At 25.0° C. the diameter of the colony reached an optimum of 8.3 cm. and an actual growth of 7.9 cm., and at 31.0° C. the diameter of the colony was 6.2 cm. with a total growth of 5.8 cm. The culture at 37.0° C. grew fairly well for the first ten days but after that no further growth. Transfers from these last plates to fresh medium produced no growth, hence it could be concluded that the heat finally killed the organism.

In fig. 1, the diameter of the colony in cm. was plotted against the time in days to obtain the rate of growth for each temperature. It may be seen here how the growth was affected. At optimum temperature, after the initial lag period of 1–2 days, growth proceeded in practically a straight line. With a deviation from the optimum temperature there is accordingly a decrease in the growth rate which corresponds to the temperature affecting it. At 16.0° C. we find a regular sigmoid curve which represents an initial lag period followed by a period of increased activity and finally a return to the lag period.

When the maxima points of total growth are plotted, fig. 2 (diameter of colony against temperature), we have the relationship of the total growth to the various temperatures outlined.

These results seem to indicate that the optimum growth for this organism has changed from approximately 37.5° C. in its parasitic condition to 25° C. in a saprophytic condition. This latter fact will be demonstrated in the animal inoculation experiments. It also shows that the longer an organism is kept in culture the greater will be its change in physiological phenomena to an optimum point for its changed environment.

Hydrogen-Ion Concentration.—The problem of hydrogen-ion concentration has been of interest for several years and its

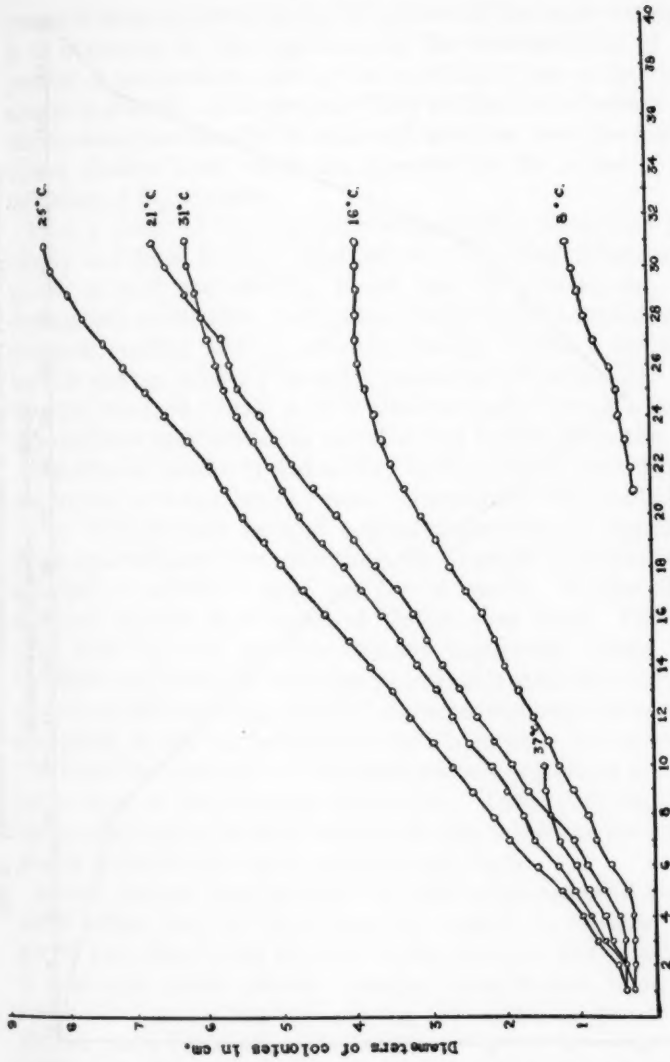


Fig. 1. Rate of growth at various temperatures on beef extract agar with same pH.

consequences especially emphasized by von Mallinckrodt-Haupt ('32). It has been demonstrated that the change of pH in a medium plays an important part in the cultural characteristics

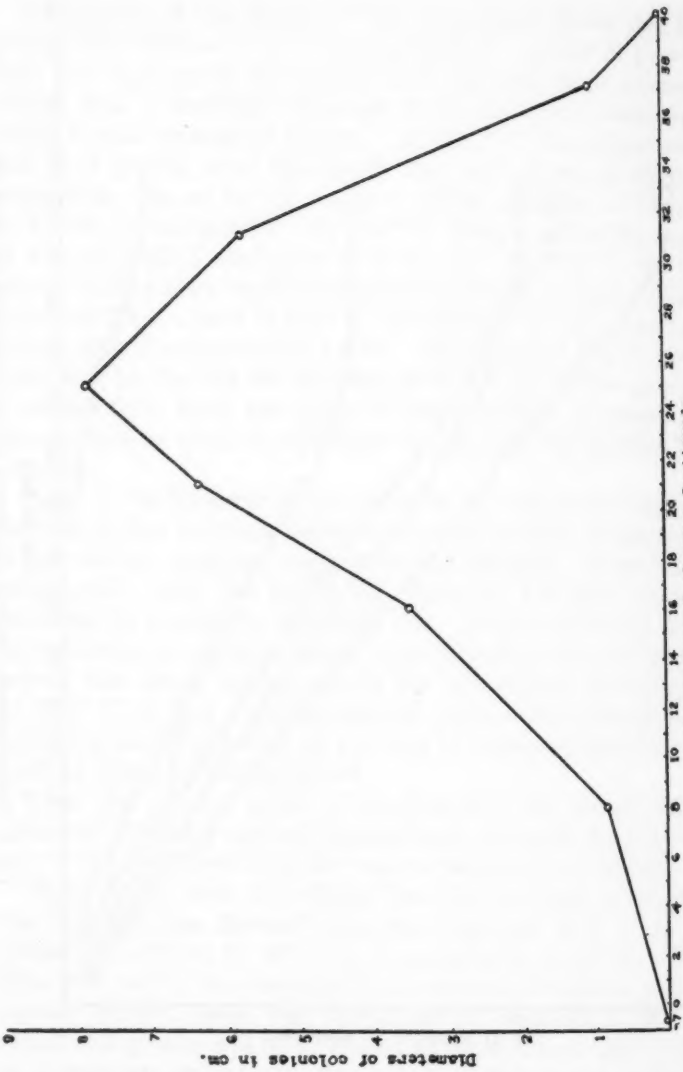


Fig. 2. Maximum growth at various temperatures on beef extract agar with same pH.

of the fungi grown. These changes may be brought about in their nutrient medium through the formation of acids which are

present in large amounts, by the breakdown of the sugar molecule in its utilization by the organism, by the decomposition of the proteins or the products, and by the cracking of fats or lipoids as a result of growth. As is perhaps fairly evident in the case of the carbohydrate reactions to be explained later, we have the formation of alkaline bases which are observed in the course of the utilization of the proteins.

From a study of the cultural characteristics mentioned previously and from further observations, a decided difference in growth on acid and alkaline media may be pointed out. In strongly acid media there is abundant budding and a formation of numerous conidia, and on strongly alkaline media a tendency towards shorter, thicker cells and a yeast-like cell formation. On the other hand, on weakly acid or alkaline media there is a favorable condition established for mycelial and hyphal formation.

The effect of alkalinity and acidity on the development of fungi was noticed for a number of years. Marantonio ('93) found that a more acid medium favored a greater quantity of mycelium. These observations were confirmed by Concetti ('00) and were extended to include a large number of media. Strains of an organism isolated from cases of thrush were used. Fineman ('21), working with *Monilia albicans* apparently, found that mycelium grew better in media under low surface tension and oxygen tension while the yeast form of the organism predominated on solid media, simple carbohydrates, and a high pH. Milochevitch ('29) found that varying the hydrogen-ion concentrations of media had no effect on the same sort of a fungus. Talice ('30) found the best development of hyphae on dilute potato decoction, the hydrogen-ion concentration being perhaps fairly high.

Several workers have pointed out that fungi possess a certain buffer action, that is, they have the ability to regulate their growth according to the reaction of the medium, and it is found to exist only under external changes. This means, then, that acid media become less acid during the course of growth and alkaline media become less alkaline.

What has been found to be true for the effect of various temperatures on the growth of *E. capsulatus* may also be said to hold with regard to hydrogen-ion concentration. For testing this,

Fig. 2. Maximum growth at various temperatures on beef extract agar with same pH.

beef extract agar made up with Liebig's extract of beef but adjusted to the various pH's shown in table II, was inoculated as described previously. The same procedure was carried out as in the above experiments, namely, daily diameter measurements and occasional microscopic examinations. Here, as before,

TABLE II

MEAN DIAMETERS IN CENTIMETERS OF COLONIES GROWN IN VARIOUS HYDROGEN-ION CONCENTRATIONS, AT A TEMPERATURE OF 26° C.

Beef extract agar (Liebig's extract of beef)													
Days after inoculation	pH values												
	2.1	3.3	4.2	5.2	5.5	6.1	6.4	7.0	7.4	7.7	8.2	9.3	10.4
1	0.2	0.3	0.3	0.3	0.3	0.3	0.3	0.4	0.3	0.4	0.3	0.4	0.4
2	0*	0	0.3	0.3	0.3	0.3	0.3	0.4	0.3	0.4	0.3	0.4	0
3	0	0	0.3	0.4	0.4	0.4	0.4	0.6	0.5	0.6	0.4	0.4	0
4	0	0	0.3	0.5	0.5	0.6	0.7	0.9	0.8	0.9	0.6	0.5	0
5	0	0	0.3	0.7	0.8	0.9	0.9	1.2	1.2	1.2	0.8	0.7	0
6	0	0	0.3	1.0	1.1	1.2	1.2	1.4	1.6	1.6	1.1	0.9	0
7	0	0	0.3	1.3	1.3	1.5	1.5	1.7	2.0	1.9	1.4	1.1	0
8	0	0	0.3	1.4	1.6	1.8	1.9	2.1	2.3	2.2	1.8	1.3	0
9	0	0	0.3	1.7	1.9	2.1	2.2	2.3	2.7	2.5	2.0	1.5	0
10	0	0	0.4	2.0	2.2	2.4	2.4	2.6	3.0	2.8	2.4	1.8	0
11	0	0	0.5	2.3	2.5	2.7	2.7	2.9	3.3	3.2	2.7	2.0	0
12	0	0	0.6	2.6	2.8	3.0	3.1	3.2	3.7	3.5	2.9	2.2	0
13	0	0	0.8	2.9	3.1	3.3	3.4	3.6	4.0	3.8	3.2	2.3	0
14	0	0	1.0	3.1	3.4	3.5	3.7	3.8	4.3	4.1	3.5	2.4	0
15	0	0	1.1	3.3	3.6	3.8	4.0	4.1	4.6	4.4	3.8	2.4	0
16	0	0	1.3	3.7	3.9	4.2	4.2	4.5	4.9	4.7	4.1	0	0
17	0	0.4	1.6	4.0	4.3	4.5	4.6	4.8	5.3	5.0	4.4	0	0
18	0	0.6	1.8	4.3	4.5	4.8	5.0	5.2	5.6	5.4	4.7	0	0
19	0	0.8	2.1	4.6	4.8	5.1	5.3	5.5	5.9	5.7	5.1	0	0
20	0	0.9	2.3	4.9	5.1	5.4	5.6	5.9	6.3	6.0	5.4	0	0
21	0	0.9	2.5	5.1	5.3	5.7	6.0	6.2	6.6	6.4	5.6	0	0
22	0	0.9	2.7	5.3	5.5	6.0	6.3	6.5	6.9	6.8	5.9	0	0
23	0	0.9	2.8	5.4	5.6	6.2	6.5	6.8	7.2	7.1	6.2	0	0
24	0	1.0	3.0	5.7	5.9	6.4	6.7	7.0	7.4	7.2	6.4	0	0
25	0	1.0	3.1	5.8	6.1	6.6	6.9	7.2	7.6	7.4	6.5	0	0
26	0	0	3.2	5.8	6.3	6.8	7.0	7.4	7.8	7.6	6.7	0	0
27	0	0	0	5.9	6.4	6.9	7.1	7.5	8.0	7.8	6.9	0	0
28	0	0	0	6.1	6.5	7.0	7.2	7.6	8.2	8.0	7.0	0	0
29	0	0	0	6.2	6.6	7.1	7.3	7.7	8.3	8.1	7.1	0	0
30	0	0	0	6.3	6.7	7.1	7.4	7.7	8.4	8.2	7.1	0	0

*0 indicates no growth

contaminations were present, so that only three plate measurements were incorporated in the table. The mean results in table II represent a typical set of data in this work.

An analysis of the data shows that no growth took place at a pH of 2.1 and 10.4. At pH 3.3, growth was not evident until the seventeenth day, when the diameter was 0.4. The total growth was 0.7 cm. on the twenty-fourth day, after which it stopped. A transfer of the culture to a medium of pH 7.2 gave normal good growth. With an increase in pH to 4.2, growth was at first slow, but the colony attained a diameter of 3.2 cm. on the twenty-sixth day and then growth stopped, showing a total increase of 2.9 cm. In like fashion, by subtracting the initial inoculum measurement from the final colony diameter, we find that at pH 5.2 total growth for thirty days was 6.0 cm.; for pH 5.5, 6.4 cm.; for pH 6.1, 6.8 cm.; for pH 6.4, 7.1 cm.; for pH 7.0, 7.3 cm.; for pH 7.4, we find the optimum pH with a total growth of 8.1 cm.; for pH 7.7, 7.8 cm.; for pH 8.2, 6.8 cm., which is the same as that for pH 6.1; for pH 9.3, total growth of 2 cm. was acquired on the fifteenth day, with the colony showing no additional growth.

By plotting the diameter of the colony against the time in days, fig. 3, we obtain the rate of growth for the optimum pH which shows, except for the initial lag and a final lag, the latter being due perhaps to external factors as slight drying and probable utilization of most of the nutrients, an almost straight line. This varies with the pH, showing a decreased growth rate from the optimum, with an increased lag and decreased period of activity.

By plotting the maxima points of growth or total growth for each pH against the pH values, we obtain a clear relationship of the growth at various hydrogen-ion concentrations, with the point of optimum growth forming a peak, as seen in fig. 4.

In addition, the pH of several of the media after growth was determined. No definitely accurate results were obtainable due to the dried-up condition of several of the media, but indications seemed to be that there was an increased pH where growth was best, with no change in the strongly alkaline and strongly acid. The results may be interpreted by considering the amount of growth as an index of the production of alkali due to the utilization and decomposition of the protein substances in the medium.

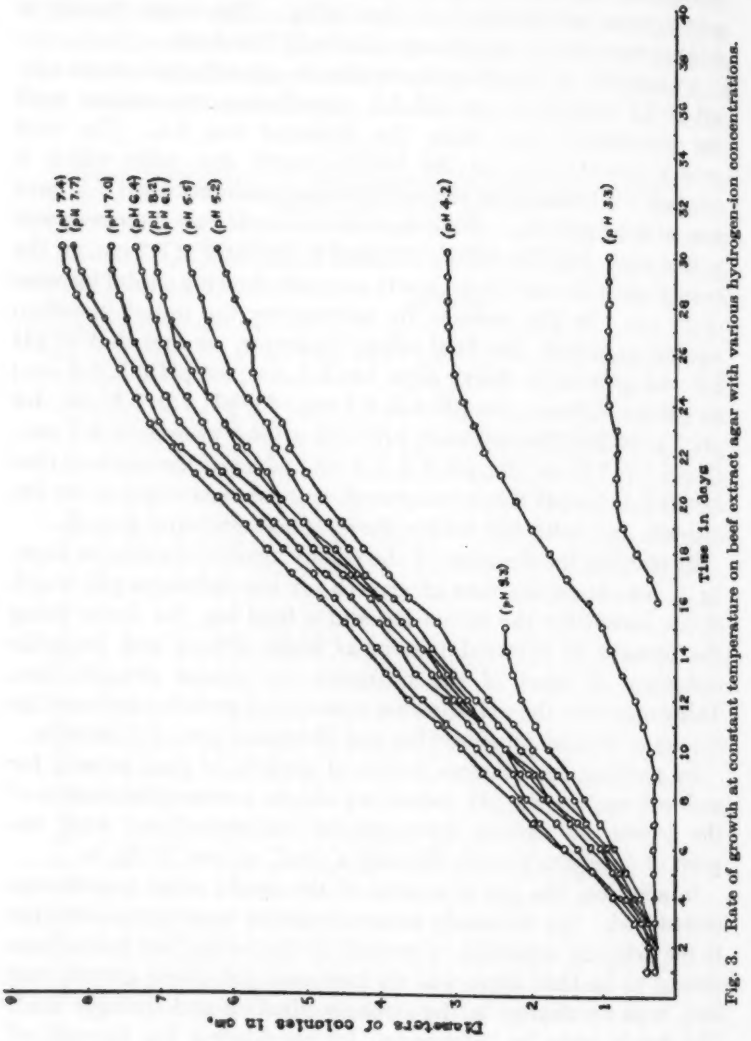


Fig. 3. Rate of growth at constant temperature on beef extract agar with various hydrogen-ion concentrations.

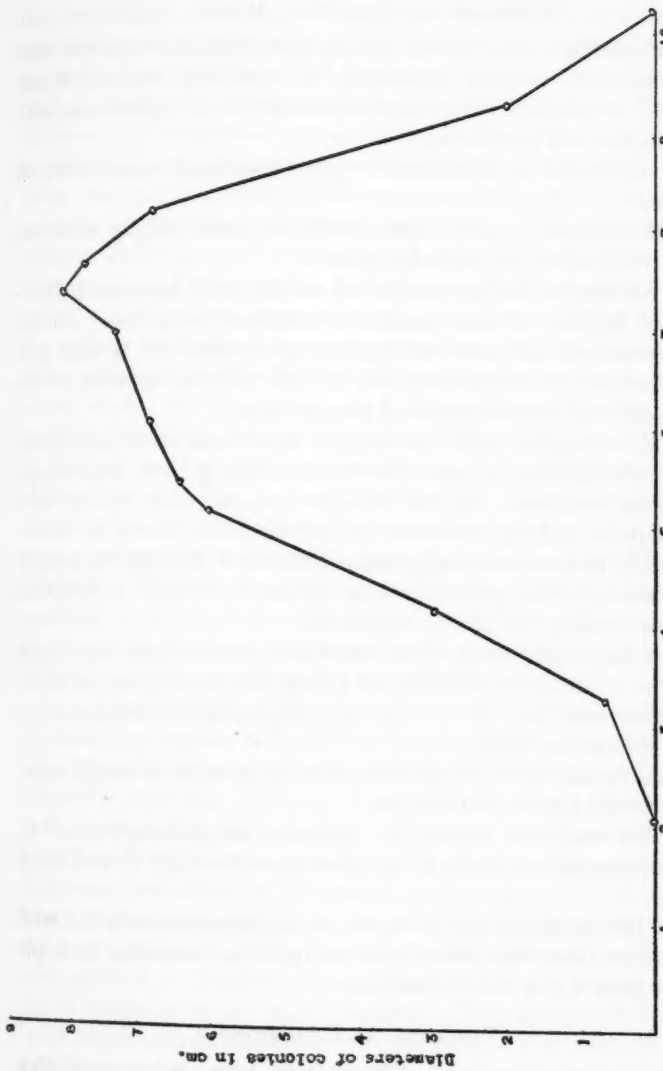


Fig. 4. Maximum growth at constant temperature on beef extract agar with various hydrogen-ion concentrations.

Fig. 3. Rate of growth at constant temperature on beef extract agar with various hydrogen-ion concentrations.

Summary of Cultural Work

The results of the cultural studies described above for the three organisms, *Endomyces capsulatus*, *E. capsulatus* var. *isabellinus*, and *E. dermatitidis*, but more especially for *E. capsulatus*, may be summarized as follows:

1. Ability of the organisms to grow on a fairly wide variety of media.

2. No growth or very little growth on media lacking or having very little protein or carbohydrate.

3. An increase in the number of conidia and decrease in diameter of hyphae on acid media as compared with fewer conidia and increased thickness of hyphae on media with a high pH. The number of conidia and size of cells may be an index of the hydrogen-ion concentration of the medium.

4. *E. capsulatus* and its variety *isabellinus* show an almost direct change from a yeast-like to mycelium growth macroscopically, as evidenced by the change from a moist colony to a filamentous colony, whereas *E. dermatitidis* shows a macroscopically evident secondary stage in the form of a definite, prickly or coremium-like growth. The secondary stage is identical microscopically, for all the organisms.

5. *E. capsulatus* has a white color, *isabellinus* a light chamois or isabella, while *E. dermatitidis* has a dark brown or cinnamon color.

6. Size and number of various morphological characteristics differ on various media.

7. Reversion to the yeast-like form by growth on serum agar.

8. No growth on litmus milk.

9. The organism shows an optimum temperature of 25° C. with no growth occurring at temperatures above 37° C. and below 8.0° C.

10. The optimum pH is shown to be approximately 7.4 with deviations from the mean, and no growth occurring in a pH higher than 9.3 or lower than 3.3.

Biochemical Reactions

Carbohydrate Reactions.—There have been many conflicting results in the past with regard to the carbohydrate reactions of yeasts and yeast-like organisms. Systems of classification have

been established whereby an organism was placed in a definite taxonomic position because of its production of either acid or gas, or both, or whether or not it had an effect on a carbohydrate. Such a system has been established by Castellani for *Monilia*. Whether any confidence can be placed in a method of that sort is doubtful, first, because it is problematical, at least in some cases, whether absolutely pure sugars are obtainable, and small amounts of impure substances may bring about an altered reaction which may not coincide with the specified phenomenon. That is, a reaction may be indicated as slight or weak, which actually should have been negative. Dekker in 1931 outlined the various methods of sugar fermentation and the faults to be found with each, and for further information, the reader is referred to her work. In the second place, it has been evidenced in the past and frequently noticed at present, that pathogenic organisms of the sort mentioned above have often lost their fermentative abilities on standing in artificial media. The constant subculturing has resulted, as in the case of many fungi, in a change from a pathogenic and virulent parasite to a non-harmful saprophyte, and while this may not necessarily be an index of the sugar reactions of an organism, still we must consider such occurrences as of some significance. It would seem, therefore, that not much reliance must be placed on a system of that sort.

In the work carried out here, stress was laid not so much on the desire to organize and establish a new method of classification, such being altogether too numerous, but to determine whether a yeast-like organism, such as *E. capsulatus*, could produce any acid or gas. For this purpose, Pfanstiehl sugars, which have been found to be considerably above the average, were used. The sugar was added, at the rate of one per cent, to a beef extract broth as prepared previously, to which had been added phenol red as an indicator. The phenol red was made up as a 0.02 per cent solution in distilled water and added in the amount of 1.5 cc. of indicator to 10 cc. of medium. This gave a fairly deep color which proved satisfactory in the work. This particular dye was chosen because preliminary experimentation had shown that the reaction tended more towards alkalinity. Litmus was also used in several series, but the results were not so clear and definite as with phenol red.

A 12-day-old culture on beef extract agar was mashed up with a sterile needle and 5 cc. beef extract broth of the same pH were added to form a suspension. A loopful of this supension was inoculated into Smith fermentation tubes which contained the above medium. All the tubes were grown at 25° C.

The carbohydrates used were those listed in table III. For the sake of clarity, they may be outlined as follows:

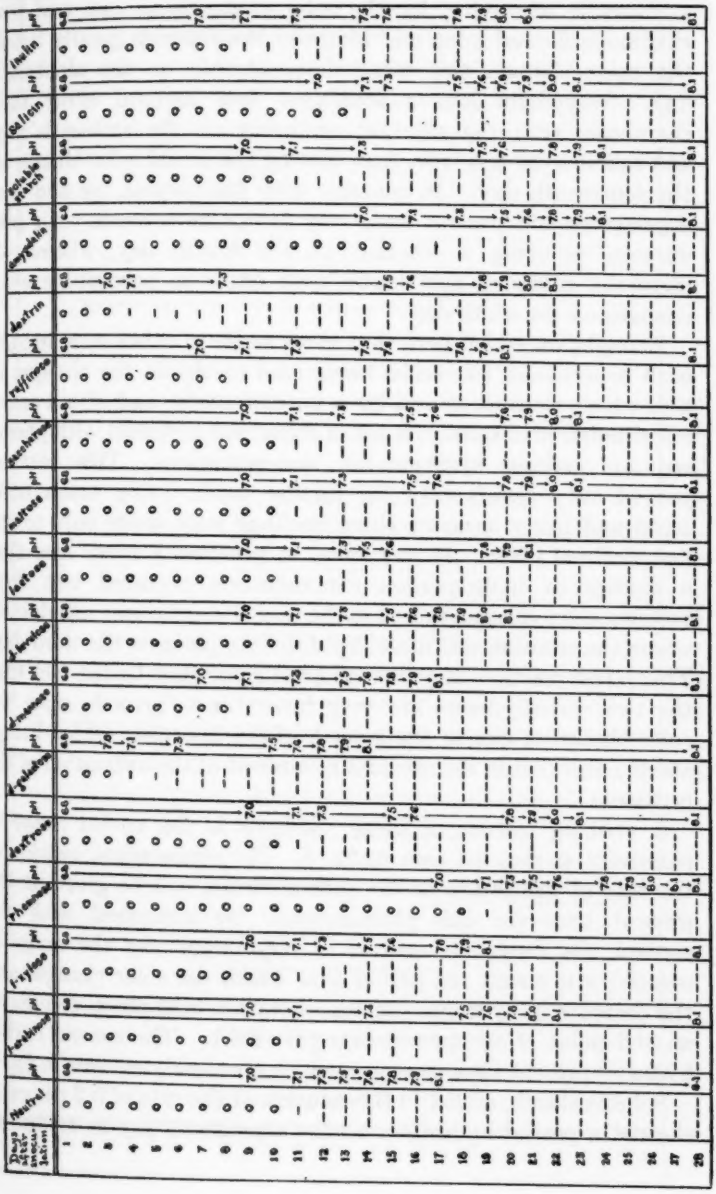
Monosaccharides.
  Pentoses:
    Aldoses;
      l-arabinose, l-xylose
  Hexoses:
    Aldoses;
      rhamnose, dextrose, d-galactose, d-mannose
    Ketose;
      d-levulose
Disaccharides.
  lactose, maltose, saccharose
Trisaccharides.
  raffinose
Colloidal polysaccharides.
  dextrin, starch (soluble), inulin
Glucosides.
  amygdalin, salicin

These carbohydrates represent a fairly wide range and include those sugars which are used in the regular routine sugar reactions, as xylose, dextrose, maltose, and lactose.

There will be no attempt at present to explain the work in detail or to offer any complex chemical formulae for possible decompositions, but the reactions and a possible empirical reason for the observations will simply be pointed out.

An examination of table III in which the carbohydrates are arranged in the order of their increasing groups, shows that when compared with the control (the same medium minus the carbohydrate) in the first column there was a changed reaction due to the presence of a carbohydrate. It will be noticed that the control tube showed a change on the eleventh day and a period of reactivity for six days, reaching its maximum on the seventeenth day after inoculation. It is further seen that 1-arabinose, 1-xylose, dextrose, d-levulose, lactose, maltose, saccharose, and soluble starch showed the same initial period of definitely de-

TABLE III
CARBOHYDRATE REACTIONS



monstrable reactivity, by the color changing to red, and that rhamnose showed a lag and hindered the reaction greatly before the color change, this being demonstrable on the nineteenth day. Amygdalin had a somewhat less harmful effect than rhamnose, with the reaction occurring on the sixteenth day, and salicin even less than that, having the initial color change on the fourteenth day. In contrast with the normal, we find that d-galactose and dextrin were very favorable towards color production, inducing a reaction on the fourth day, whereas d-mannose, raffinose, and inulin took effect in perceptible color changes on the ninth day.

It might be added here that three series of tubes were used in each experiment, two series being used to obtain the changes in pH, which were measured by a potentiometer and also a set of colorimetric standards. A set of tubes was prepared with phenol red at various hydrogen-ion concentrations. This method proved very satisfactory for further work, being much more rapid and fairly accurate since readings were made only to the first decimal place. It was found in practically every case that a change in hydrogen-ion concentration occurred two days before a color change, except in the case of d-galactose and dextrin where the reaction was more rapid, taking place on the third day. This latter reaction may be explained somewhat by the fact that the two carbohydrates are very favorable to growth, since the color change is due to the growth of the organism which brings about a reaction in the medium as a result of the utilization of the nutrients.

A striking feature of these reactions is the varied range of reactivity as may be seen in fig. 5. No explanation, outside of the rate of utilization of the carbohydrate, can be given at the present time for this phenomenon. As compared with the control, the range for dextrin is very large. By the range of reactivity is meant the period over which the color changes and the decrease in hydrogen-ion concentration take place, coming to an end point of an approximate pH of 8.1. The various carbohydrates present various ranges which are clearly evident in fig. 5.

Soluble starch, added to the solution at the rate of 0.2 per cent, showed a partial hydrolysis on the nineteenth day as evidenced

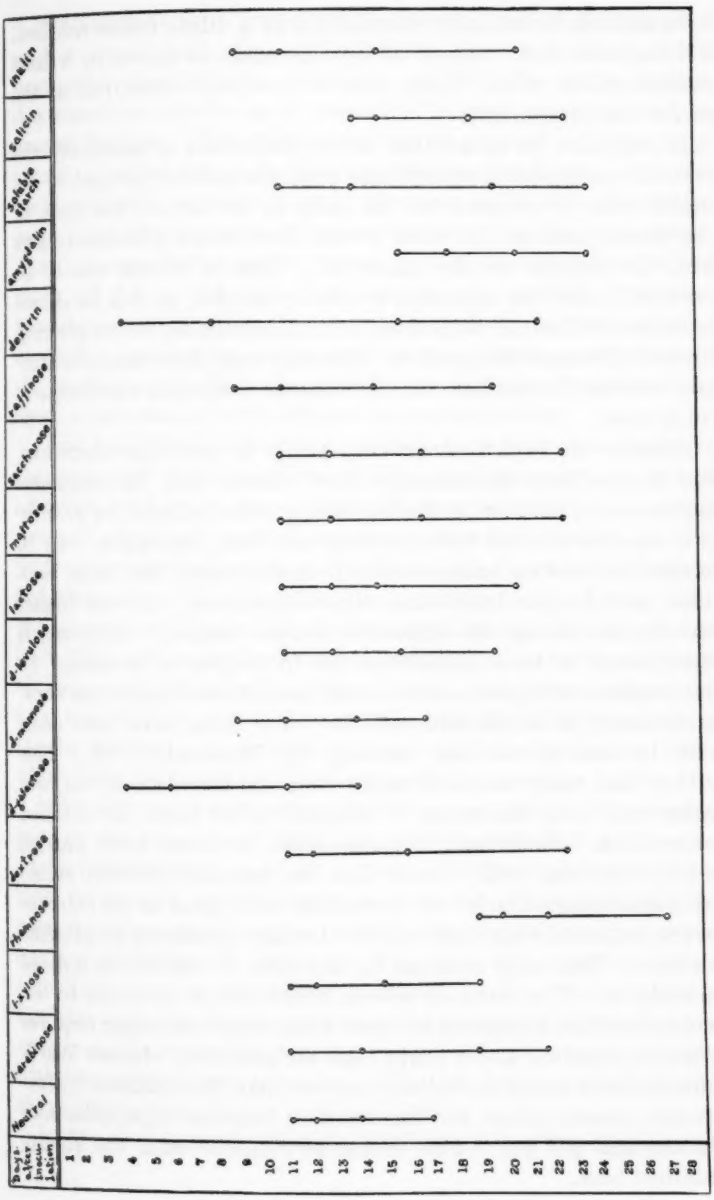


Fig. 5. Range of reactivity of carbohydrates.

by a reddish-brown color on addition of a dilute iodine solution. Fehling's solution reduced to cuprous oxide as shown by a faint reddish-violet color. There was no complete hydrolysis at the end of the experiment.

It may also be noted that in its production of alkalinity the reaction proceeded in every case from the aerobic portion of the Smith tube, or rather from the bulb, to the arm of the tube, or the closed portion. In other words, there was a diffusion of ions from the aerobic to the anaerobic. This of course was to be expected, since the organism is strictly aerobic, as will be shown later, and utilized all of the little material available, before growing towards the anaerobic portion. In every case, however, no matter how marked the reaction, the color change in the arm was comparatively slow.

Proteins are broken down very easily by reacting substances, and the cultural experiments have shown that *E. capsulatus* requires some protein, its derivatives, or carbohydrate, for growth. It is also known that these proteins and their derivatives may be further reduced or broken down to amino acids, peptones, peptides, and hexone bases and other substances, whereas higher carbohydrates may be reduced to simpler sugars. Ammonia is often found to be a product in the hydrolysis of proteins. In the presence of sugars, amino acids have characteristic reactions, particularly in an alkaline solution, and these have been dealt with by several authors, recently by Watanabe ('32). This author lists many reactions and covers the literature of the field rather well, and the reader is referred to his paper for detailed information. Euler and his associates, in some work carried out in 1926 and 1927, found that the reaction between amino acids and sugars which were reversible, took place at an equimolecular ratio and that their velocity became greater in an alkaline medium. This may account for the rate of activity as noticed in table III. The work of several others seems to point to the conclusion that a reaction between amino acid and sugar requires a neutral reaction and a fairly high temperature, whereas Waldschmitt-Leitz and Rauchalles observed that the optimum hydrogen-ion concentration for the reaction between dipeptides and glucose was pH 8.1. This seems to coincide with the results obtained here.

It is clearly evident from the experimental data that, instead of acid or gas or both, the carbohydrates here used induced the production of an alkaline condition. In addition to the protein decomposition substances as mentioned above, ammonia may be an end product. This substance, where present in fairly large amounts, brings about a strongly alkaline condition. To test for it, several cc. of the culture broth were poured into a test-tube, and a pinch of anhydrous sodium carbonate (Na_2CO_3) was added. The tube was shaken and a rather strong odor of ammonia was given off. It is possible that, besides the evident ammonia, hexone bases, as arginine, lysine, and histidine, which are alkaline in solution, are formed as products of the protein derivatives in the medium and that in addition to the sugar reaction they cause a decrease in the hydrogen-ion concentration.

Indol and Skatol.—Reactions which have long been used in bacteriological technique but which have not received much attention until lately, due to the additional knowledge and better methods which are applied, are those of indol and skatol. The work of such investigators as Pittaluga ('08), Goré ('21), Holman and Gonzales ('23), Morelli ('09), Sasaki ('10), Zipfel ('12), Krumwiede and Pratt ('13), Fellers and Clough ('25), Koser and Galt ('26), and several others has greatly added to the methods of technique in investigations. Tests have been advocated from time to time, the importance of which lay in their specificity for the reaction. Fellers and Clough list about thirteen tests which have been applied, some successful some not.

Indol and skatol are protein derivatives and their determination rests on the presence of tryptophane. From it they are broken off by a deamidization to form first indol proprionic acid, then indol acetic acid, and finally indol or skatol. The medium to be used in such a reaction must therefore contain a supply of tryptophane sufficient to determine whether the organism would actually be capable of breaking it down to indol or skatol or both. For this purpose, it was necessary to use peptone plus 0.1 per cent casein in water to insure good growth and also to comply with the requirements as stated. The broth was inoculated and the organism was allowed to grow at 25°C.

In the first test the Gnezda ('99) oxalic acid principle was

applied. Strips of filter paper were placed in a warm solution (saturated) of oxalic acid. On cooling, crystals of oxalic acid remained on the strips. The paper was then dried well and inserted in the mouth of the flask under aseptic conditions, so that it was pressed against the side of the flask and just above the surface of the broth. If indol is formed it volatilizes and colors the oxalic acid pink. In these tests for *E. capsulatus*, a very faint pink was barely discernible. This test has been advocated rather strongly by the Society of American Bacteriologists for regular routine work in bacteriology.

A test that was applied successfully is the Ehrlich-Böhme technique which consists of two solutions. Solution 1 contains 1 gm. para-dimethyl-amino-benzaldehyde, 95 cc. ethyl alcohol (95 per cent), and 20 cc. hydrochloric acid, concentrated. Solution 2 is a saturated solution of potassium persulphate ($K_2S_2O_8$). In the experiment, 5 cc. of solution 1 are added to 10 cc. of the culture fluid, then 5 cc. of solution 2, and the mixture is shaken. A faint red color appearing about five minutes later indicated a weakly positive reaction. This method has proven rather successful for indol. Steensma ('06, '06a) substituted vanillin for the para-dimethyl-amino-benzaldehyde, but this was not applied here.

The Salkowski ('83) nitroso-indol test was negative for indol.

Skatol was tested for by the Sasaki ('10) methyl alcohol test. This method consisted of adding to the broth to be tested about four drops methyl alcohol and an amount of concentrated sulphuric acid equal to the broth. The acid contained a trace of ferric salt. A reddish-violet color indicated a positive skatol reaction.

Under the proper conditions, indol and skatol may be produced by *E. capsulatus*.

Relation to Free Oxygen.—Aerobiosis has often been considered important in experimental work. Organisms are classified as strict aerobes, facultative aerobes, or facultative anaerobes and strict anaerobes. In order to find the position of *E. capsulatus* in this classification, tubes of broth were prepared and inoculated by the Liborius method (Zinsser, '29) and kept at 25° C. Cultures were kept at the same temperature and in the

same broth, but were plugged only, allowing oxygen to enter. No growth occurred in the former while a luxuriant growth occurred in the latter. Agar stab cultures were negative for growth in the stab, but positive on the surface. The relationship to free oxygen was also demonstrated in the carbohydrate reactions as mentioned previously.

We may conclude, therefore, that *E. capsulatus* is a strict aerobe, at least in so far as its growth on agar after a period of time has demonstrated. When in the yeast form, anaerobic cultures showed slight growth, a condition of facultative anaerobiosis. Continued growth on an artificial substrate has converted it into a strict aerobe.

Reduction of Nitrates.—The tests for nitrate reduction as advocated by the American Society of Bacteriologists were inconclusive and unsatisfactory. Ammonia was found to be present, but that was apparently due to the breakdown of the peptone and other protein decomposition products. Trommsdorf's method was also indefinite, being negative in general, but at times gave a slightly colored reaction.

Production of Hydrogen Sulphide.—The formation of hydrogen sulphide in culture has often been concerned with a toxic action of the lead salt or lead acetate in the medium toward certain bacteria. Whether hydrogen sulphide would be produced here was of course problematical. However, a lead acetate agar put up by the Digestive Ferments Co., "Bacto lead acetate agar," which is claimed to contain too little lead salt to be toxic and yet was favorable for the demonstration of the hydrogen sulphide, was used. Making stab cultures was of course illogical, for reasons pointed out before, and so surface inoculations were made and the cultures incubated at 25° C. A slight brownish coloration was found to occur directly around the colony after thirty to forty days, and increased somewhat on standing.

No definite method has been advocated for the above experiment, and since the results were slightly evident even though the period was a great deal longer than for bacteria, the reaction might be considered positive.

Gelatine Liquefaction.—Tubes of 15 per cent meat extract gelatine were inoculated by stabbing and incubated at 25° C. A

slow liquefaction took place between 30 and 40 days, and in some cases even longer than that. Liquefaction began at the surface and proceeded downward. This may be correlated with the growth of the fungus.

Reaction to Light

A great deal of experimental work has been done in the past, and much is being attempted at the present, with respect to the

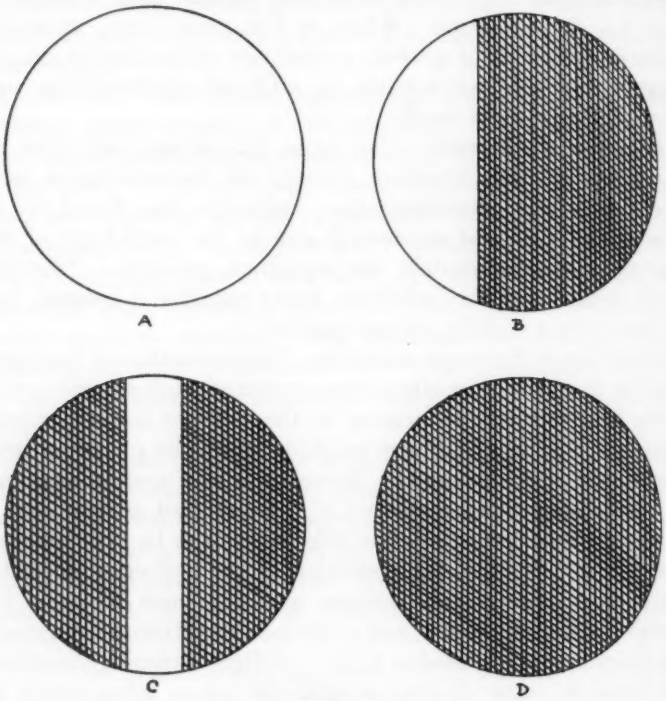


Fig. 6. Explanation in text.

action of light on higher plants and also microbiology. It is accepted as a fact by many that plant growth and vitality can be greatly altered, either for better or worse, by continued treatment under variously colored lights. No great amount of work, however, has been done on the action of the primary colors on fungi or bacteria. Several workers have found a destructive

action of light on the lower organisms, and Rahn has found that red and green light have no effect while blue light has a very harmful action, with the degree of destruction decreasing in the violet and ultra-violet portion of the spectrum.

Effect of White Light on Growth.—To determine the differential action of light and darkness on *E. capsulatus*, series of plates were

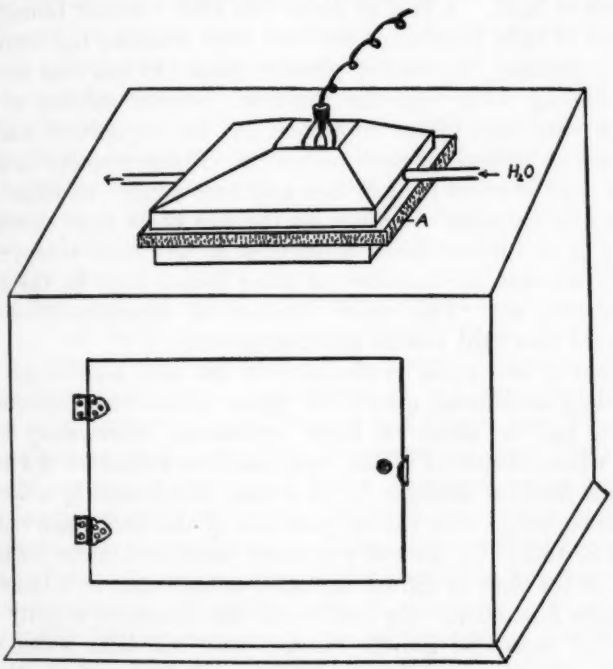


Fig. 7. Explanation in text.

covered with opaque black paper of the sort used in covering photographic plates, as illustrated in fig. 6, the portions with the crossing lines representing the covered areas. The plates were treated as follows: plate A was inoculated in the center and completely exposed to the light; plate B was covered as shown and was inoculated at a point about 1.5 cm. from the rim of the plate, or about 5 cm. from the uncovered area. This plate was used to determine whether the organism would grow towards the

light or not. The plates covered as in C were inoculated in the center of the portions covered, and plate D was inoculated as in plate A and served as the complete darkness plate. Nutrient agar was used. These plates were placed in a light-proof container illustrated in fig. 7, at room temperature. A General Electric mazda bulb of 50 watts and 115 volts was used as a source of light. A flow of water was kept constant beneath the source of light to prevent the heat from reaching the interior of the container. A sheet of colorless glass (A) was kept between the flowing water and the cultures. Several cultures of each series were kept in the container and the experiment was performed on several different occasions. Plates prepared as above were kept at room temperature and were subject to diffuse daylight and darkness. Growth at the end of 24 days showed no cultural or microscopical differences in any case, except for a slight increase in diameter of three plates kept in the freely circulating air. This small number of attempts seemed to indicate that light had no effect on growth.

Effect of Red Light on Growth.—It has been pointed out that red light stimulated growth in higher plants but, according to Rahn, had no effect on lower organisms. Shrewsbury found that when cultures of *Willia* were placed at a distance of 4 inches from a Wratten safelight No. 1 screen, illuminated by a 60-watt gas-filled lamp, with the temperature of the dark room ranging from 23 to 28° C., growth was more rapid and more luxuriant than in the dark or diffuse daylight, as expressed by a hastening of spore formation. He attributed this increased activity to a reflex of increased growth energy, with red light acting as a stimulant. However, he adds that much the same growth was obtained in cultures grown at 30° C. as in those grown at room temperature, so that it was very likely that the heat and not the red light was the activating agent.

To avoid this condition, the light-proof container as described above (fig. 7) was again used. This time a red bulb of the same intensity as before was used and a plate of red glass replaced the colorless glass. The same medium of the same pH was used here too. Cultures were grown at room temperature in the dark and diffuse daylight as controls. After 27 days it was found that

those cultures under red light displayed no cultural or microscopic differences from those grown in the dark and daylight, and showed no change from those in white light and total darkness, except for a small increase in diameter which may be ascribed to the extra time of 3 days.

Reaction to Dyes

The rôle of certain dyes in medical work has received much critical and experimental attention for a number of years. Their application for therapeutic purposes has resulted in clinical cures in various cases and has had no effect in others. Particular dyes, as methylene blue, gentian violet, and crystal violet, have been used locally or intravenously for skin lesions in cases of blastomycosis, coccidioidal granuloma, sporotrichosis, monilial infections, and other diseases both of mycological and bacteriological etiology. Some of these have yielded favorable results as mentioned, but the value of the dyes applied is empirical since dye therapy rests on no definite scientific basis, according to Spring ('29). Faber and Dickey ('25) treated 15 infants afflicted with thrush, with local applications of a 1 per cent aqueous solution of gentian violet and found that 50 per cent showed an apparent cure in one day or less; 36 per cent in from 2 to 3 days; and 14 per cent in from 4 to 5 days. In several cases, however, the lesions returned at various intervals, and again some disappeared after one application while others resisted for several applications. In general, the lesions seemed to become smaller after continuous applications and to disappear. As a result, Faber and Dickey advocate a trial of the dye for prophylactic measures and also for therapy. Churchman ('20d), working on chemotherapy with gentian violet *in vitro*, advised careful considerations of the amount of bacteria affected, before any conclusion as to the therapeutic value of the dye be evaluated. Sanderson and Smith ('27) postulated the possibility of the utilization of gentian violet dye for the treatment of blastomycosis.

It has been known and recorded since the days of Koch that certain aniline dyes have a "bacteriostatic" effect on the culturability of various bacteria. The viability of the organisms is

diminished to such an extent that they lose their power to multiply. This selective action of the dyes is very remarkable, certain organisms growing very abundantly and luxuriously on a medium containing gentian violet in a rather high concentration, whereas other organisms which are very resistant to harmful factors do not grow at all. This high selectivity or specificity was attributed not only to gentian violet, but also to others, as crystal violet and various members of the tri-phenyl methane series of dyes.

In early work on dyes, smears and broth cultures were used, but it was found that the bacteria ceased growing or were killed in the smears and that various concentrations of dye in the broth had practically the same effect. Later work (Churchman, '12) was performed on the divided agar plate to determine whether the reaction would be the same. This method consists of inserting a metal strip (Halsted's aneurysm metal) across the diameter of the petri dish and then pouring the agar on one side and allowing it to cool. The agar containing the dye is poured on the other side of the strip. When the agar hardens, the metal strip is lifted out with sterile forceps. Thus one half of the plate contains plain agar and the other half the dye-containing agar. This method has been used by several investigators, including Sanderson and Smith.

Churchman ('12,' 20, '20a, '20b, '20c, '20d, '20e, '21) found that a parallelism with the reaction of the Gram stain could be made. That is, considering as violet-positive those organisms which were inhibited in growth by a certain concentration of a dye, gentian violet for instance, and as violet negative those upon whose growth the dye had no effect, then it was found that a great number of Gram negative organisms were also violet negative. This is a general rule, with a few outstanding organisms being exceptions.

The same author also found that the selective action of the dye may be expressed in one of two ways. The dye may be toxic to the violet-positive organisms when a direct application is made, or a very strong inhibitory action is expressed when it is incorporated in the medium. On the other hand, the dye is not toxic to violet-negative organisms, and further, no inhibitory

action is expressed except in very strong concentrations when it is incorporated in the medium. His experimental evidence pointed to a dilution of 1 : 1,000,000 as capable of stopping growth, and 1 : 2,000,000 as possibly retarding it.

This work was confirmed by Krumwiede and Pratt ('13), who used dahlia agar, and also they confirmed the belief that the action was quantitative. They further observed that several dyes, in addition to those already mentioned, had this specific action. This work then called forth several applications of this principle to culture media, as that of Petroff ('15) which was used by that author to isolate tubercle bacilli from the sputum and feces, and that of Farley ('20) as a restrainer in the isolation of pathogenic molds. Farley found that a dilution of 1:500,000 inhibited the growth of Gram positive bacteria.

Later work on this problem showed that there might exist two types of organisms within a single strain, one which might grow vigorously on gentian violet media, and the other not at all, yet both showing the same stain and cultural characteristics. Also, heavy and repeated inoculations of violet-positive organisms would give fair and even good growth, and on this point Churchman ('20e) made the following statement: "This would indicate that bacteria do not, as is commonly supposed, act as isolated individuals; they possess the power, in numbers, of accomplishing effects which, alone, they are incapable of. The nature of this community of action it is at present impossible to guess at." The same author (Churchman, '21) placed dead bacterial bodies between living bacteria and gentian violet media and found that the Gram positive organisms grew, the dye having no effect. He attributed this occurrence either to a filtration or a stimulation of growth. The same year Churchman and Kahn ('21) found that a number of cells could accomplish more than a single cell, hence the communal action belief was further emphasized.

Work on dyes was not confined to bacteria alone, for Lewis ('30) found that a certain number of dyes failed to inactivate the virus of chicken tumor while many had a harmful effect in certain concentrations. Several papers have been published showing the action of certain of these dyes on various fungi, but only in a qualitative way; Greenbaum and Klauder ('22) tested the action of gentian violet

as advocated by Farley ('20a); Sanderson and Smith ('27) found that dilutions up to 1 : 500,000 inhibited *E. dermatitidis* in its yeast-like form; Clark ('27) showed that no growth occurred in a yeast-like organism and its mycelial form in dilutions from 1 : 100 to 1 : 25,000 and that it gradually increased from 1 : 50,000 to 1 : 1,000,000, which gave good growth. No quantitative determinations of the colony growth were made. Stearn and Stearn ('29) found that several fungi, including *E. dermatitidis*, were inhibited at various concentrations of gentian violet, with the organism being affected at 1 : 500,000.

The later work of Churchman indicated that the "bacteriostatic properties" of dyes ascribe to them the ability to interfere with the reproductive mechanism, which he terms "genesistasis," without killing or in any way interfering with their other properties. This same phenomenon has been found both qualitatively and quantitatively to hold for *E. capsulatus*.

Since the author could find that no particularly quantitative results had been obtained for ascomycetous fungi, at least for the hyphal form, it was decided to investigate the problem of the dye reaction. For this purpose, several dyes, picked at random, were used. These are as follows:

Dyes of the nitro, azo, and oxyquinone groups.
  Dyes of the azo group:
    orange G—acid—(National Aniline and Chemical Co.)
    Sudan III—weakly acid—(E. Merck)
  Dyes of the oxyquinone group:
    alizarin—acid—(E. Merck)
The quinone-imide dyes.
  The thiazins:
    methylene blue—basic—(Coleman and Bell Co.)
  The azins:
    Amido-azins or eurhodins;
      neutral red—weakly basic—(Coleman and Bell Co.)
    Safranines;
      safranine A—basic—(Coleman and Bell Co.)
The phenyl-methane dyes.
  Tri-phenyl methane derivatives:
    Di-amino tri-phenyl methanes;
      light green S. F. yellowish—acid—(Coleman and Bell Co.)
    Tri-amino tri-phenyl methanes (rosanilins);
      basic fuchsin—basic—(Coleman and Bell Co.)
      crystal violet—basic—(Coleman and Bell Co.)
      aniline blue—acid—(National Aniline and Chemical Co.)

The xanthene dyes.
Fluorane derivatives:
eosine B—acid—(Coleman and Bell Co.)
Phenolphthalein and the sulphonthaleins:
phenol red or *phenol-sulphonphthalein*—acid—(Dr. T. Schuchardt, G. m. b. H. Chemische Fabrik)
The natural dyes
Brazilin and haematoxylin:
haematoxylin (Coleman and Bell Co.)

These dyes were incorporated in the media, nutrient agar (product of Digestive Ferments Co.) at a pH of 6.8, in the following concentrations: .00001; .000025; .00005; .000075; .0001; .00025; .0005. Several plates were used for each concentration of the dye, but only three plates for each are recorded in the tables for reasons stated previously. These plates were inoculated in the same manner as were those in the temperature and pH experiments, a broth of pH 6.8 being used here. The plates were kept at room temperature, which showed slight changes from day to day but not enough to affect the results seriously. The diameters of the colonies were measured daily at approximately the same hour each day. Inasmuch as the colonies may show slight irregularities in peripheral growth, these measurements may not be considered absolutely correct. However, if the shape of the colony was irregular, several diameters were measured and the mean taken as representative of that colony. Where contaminations occurred, as has been explained previously, the plates were discarded.

It is to be noted that these experiments were carried out on several different occasions and that the figures in the tables denote the mean of a representative series. Since preliminary experiments with these dyes had given some seemingly strange results, microscopic examinations were made daily, in the case of the crystal violet and methylene blue series, and then, some time after growth had started, in the case of eosine B.

Several of the dyes produced no effect on the growth of the organism. These dyes were orange G, Sudan III, alizarin (table IV), safranine A, light green, basic fuchsin, aniline blue, phenol red, and haematoxylin. Neutral red was also used, but, as was expected, the results showed no difference from the normal control, the same being true for the other dyes.

If normal conditions are maintained for the plates, that is, if there is no drying out and no change in temperature the colonies grow at a rate of speed which is fairly constant as long as there is sufficient nutrient material for the organism. However, when the plates are kept in an atmosphere which allows drying out of the agar and when the amount of nutrient material is relatively small for the particular organism, the colony will show a decrease

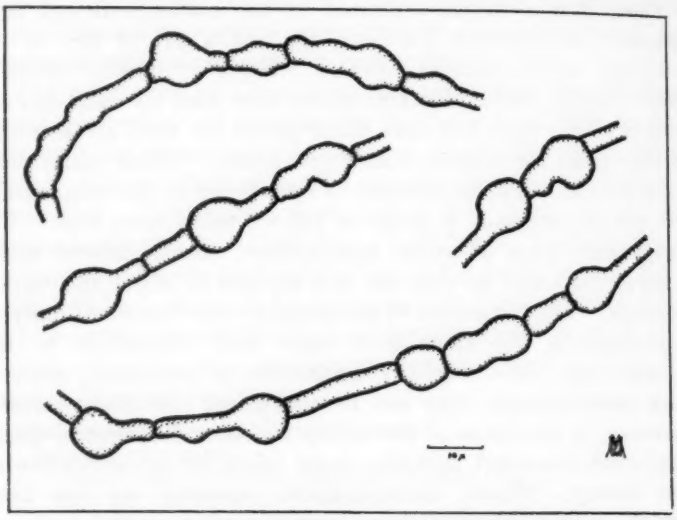


Fig. 8. Mycelium on .00025 per cent crystal violet agar.

in growth rate. Thus, in the former case, the growth curve would be a straight line for a considerable portion, depending of course on the rapidity of growth of the organism compared from day to day, which in this case was sufficient to cover the plate almost completely before drying of the substrate. An initial lag on artificial substrates, as agar, must be taken into consideration. In that case the curve would tend to be sigmoid, that is, there would be an initial lag due to an acclimatization of the organism to the medium, a portion of increased activity which would be a sharp rise, and then a portion of decreased activity due to factors mentioned previously.

The data of the growth on the several non-affecting dyes

TABLE IV

MEAN DIAMETERS IN CENTIMETERS OF COLONIES GROWN IN VARIOUS CONCENTRATIONS OF ALIZARIN, AT A TEMPERATURE OF APPROXIMATELY 22° C.

Nutrient agar (product of Digestive Ferments Co.) pH 6.8

Days after inoculation	Percentage concentration of dye						
	.00001	.000025	.00005	.000075	.0001	.00025	.0005
1	0.3	0.3	0.3	0.5	0.4	0.4	0.4
2	0.3	0.3	0.3	0.5	0.4	0.4	0.4
3	0.3	0.3	0.3	0.5	0.4	0.4	0.4
4	0.5	0.6	0.6	0.8	0.7	0.6	0.6
5	0.6	0.7	0.8	0.9	0.9	0.8	0.8
6	0.8	0.8	1.0	1.0	1.1	0.9	1.0
7	1.0	1.0	1.1	1.2	1.2	1.0	1.2
8	1.3	1.3	1.4	1.4	1.5	1.3	1.5
9	1.5	1.6	1.6	1.6	1.6	1.6	1.6
10	1.7	1.7	1.8	1.8	1.9	1.8	1.9
11	1.9	1.9	2.0	2.0	2.1	2.0	2.0
12	2.0	2.1	2.2	2.2	2.3	2.2	2.3
13	2.2	2.3	2.4	2.4	2.5	2.4	2.5
14	2.4	2.5	2.6	2.6	2.7	2.7	2.7
15	2.7	2.8	2.8	2.9	3.0	2.9	3.0
16	2.9	3.0	3.1	3.0	3.2	3.2	3.2
17	3.1	3.2	3.3	3.3	3.4	3.4	3.4
18	3.3	3.4	3.5	3.5	3.6	3.5	3.6
19	3.6	3.6	3.7	3.7	3.7	3.7	3.7
20	3.7	3.7	3.9	3.8	3.9	3.9	3.9
21	3.9	3.9	4.0	4.0	4.1	4.0	4.1
22	4.1	4.1	4.2	4.2	4.3	4.2	4.3
23	4.2	4.3	4.3	4.3	4.4	4.4	4.4
24	4.5	4.5	4.5	4.5	4.7	4.6	4.6
25	4.6	4.6	4.7	4.7	4.8	4.7	4.7
26	4.8	4.8	4.8	4.8	5.0	4.9	4.9
27	4.9	4.9	5.0	5.0	5.1	5.0	5.1
28	5.1	5.1	5.1	5.1	5.2	5.2	5.2
29	5.2	5.2	5.3	5.3	5.3	5.3	5.4
30	5.4	5.4	5.4	5.4	5.6	5.5	5.6
31	5.6	5.5	5.7	5.6	5.7	5.6	5.7
32	5.8	5.7	5.8	5.8	5.9	5.8	5.9
33	5.9	5.9	6.0	5.9	6.1	5.9	6.1
34	6.1	6.1	6.2	6.1	6.3	6.1	6.2
35	6.2	6.2	6.3	6.2	6.4	6.2	6.4
36	6.4	6.4	6.5	6.5	6.6	6.4	6.6
37	6.6	6.6	6.6	6.6	6.7	6.5	6.7

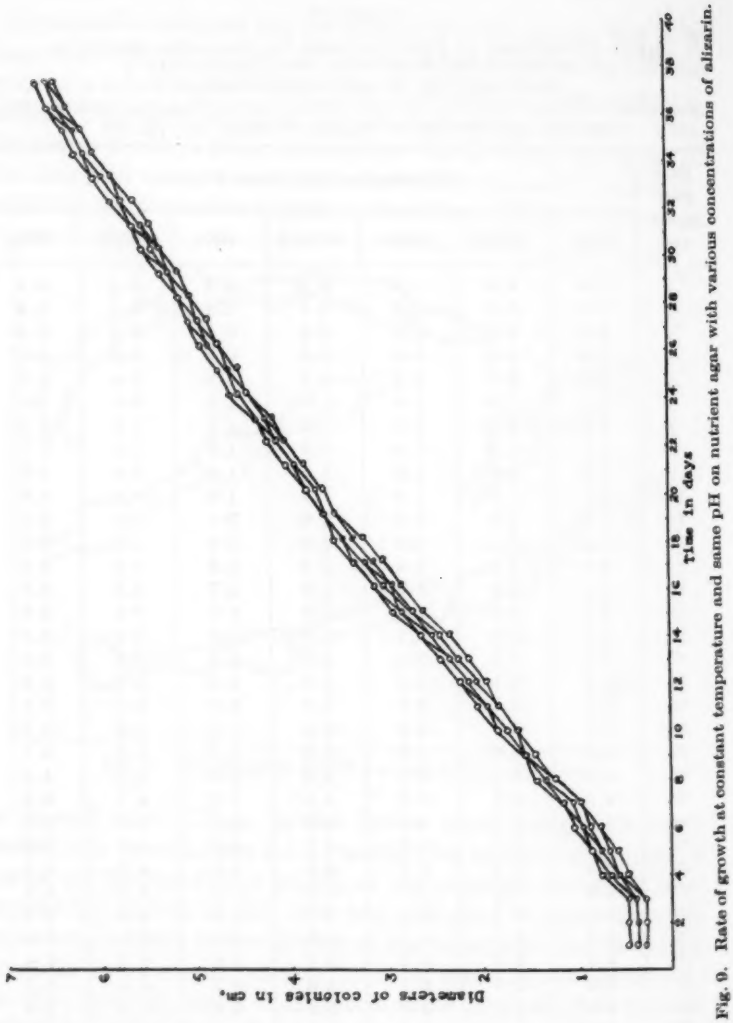


Fig. 9. Rate of growth at constant temperature and same pH on nutrient agar with various concentrations of alizarin.

showed that the rate was practically the same in each case, and if the diameters of the colonies in cm. were plotted against the time in days, for each concentration of the dye, one would find this to be the case. This is illustrated in fig. 9, the growth rate for

alizarin, results representative of these dyes. The differences in the curves are within the range of error, so that in general we may

Fig. 10. Maximum growth at constant temperature and same pH on nutrient agar with various concentrations of alizarin.

say that the growth rate is the same. Except for the initial lag and a slight curve due perhaps to utilization of nutrient materials

or drying of the agar, the line is about straight. When the maximum points of growth for each concentration is plotted, that is, diameter of colony against percentage concentration of dye (fig. 10), it is found that a straight line results, with perhaps some excusable error. In other words, growth is identical for all the concentrations of dye, at least in the observations made here.

In dealing with the other dyes, as crystal violet (table v), methylene blue (table vi), and eosine B (table vii), a striking difference in growth was observed. The cultures of crystal violet grown under the same conditions as those above presented a growth which was proportional, within a range of limits, to the concentration of the dye. Like results were found to be true for methylene blue, but not quite so well marked.

Colony measurements were made daily, and, as may be seen in table v, the growth was inhibited in the higher concentrations to the point of complete cessation. Along with the measurements, microscopic examinations were also made, and it was found that on the media of higher concentration of dye there was a corresponding decrease in production of reproductive parts. In other words, the number of asci with the ascospores gradually diminished on the increasing concentration of dye media as compared with the normal growth. A well-marked feature was the development of thick walls in the hyphae, as may be seen in fig. 8 which illustrates variously thick-walled swellings, chlamydospores and hyphae, taken from a culture of .00025 concentration, forty days after inoculation. The cellular material stained heavily, and showed granular cytoplasm and a reduction in the number of nuclear divisions. An increase in the number of swellings, cellular contents, and thickness of walls, as well as the number of chlamydospores and terminal hypnospores, is a condition which is usually associated with unfavorable phenomena. Similar characteristics were present approximately three months later. Macroscopically, the colonies on the higher concentration of crystal violet showed a growth upwards on the inoculum and not until 15 days after inoculation did any growth appear on the agar and then very little. In the case of methylene blue, however, growth was flat and took on a blue color very soon after inoculation.

TABLE V

MEAN DIAMETERS IN CENTIMETERS OF COLONIES GROWN IN VARIOUS CONCENTRATIONS OF CRYSTAL VIOLET, AT A TEMPERATURE OF APPROXIMATELY 22° C.

Nutrient agar (product of Digestive Ferments Co.) pH 6.8

Days after inoculation	Percentage concentration of dye						
	.00001	.000025	.00005	.000075	.0001	.00025	.0005
1	0.3	0.2	0.3	0.2	0.4	0.3	0.3
2	0.3	0.2	0.3	0.2	0.4	0.3	0.3
3	0.3	0.2	0.3	0.2	0.4	0.3	0.3
4	0.5	0.3	0.3	0.2	0.4	0.3	0*
5	0.7	0.5	0.4	0.3	0.4	0.3	0
6	0.9	0.6	0.5	0.3	0.4	0.3	0
7	1.0	0.8	0.5	0.4	0.4	0.3	0
8	1.2	0.9	0.6	0.4	0.4	0.3	0
9	1.5	1.1	0.6	0.4	0.4	0.3	0
10	1.7	1.3	0.6	0.4	0.5	0.3	0
11	1.9	1.4	0.6	0.4	0.5	0.3	0
12	2.1	1.6	0.6	0.4	0.6	0.3	0
13	2.3	1.8	0.7	0.5	0.6	0.3	0
14	2.5	2.0	0.7	0.5	0.6	0.3	0
15	2.9	2.1	0.7	0.5	0.6	0.4	0
16	3.0	2.2	0.8	0.6	0.6	0.4	0
17	3.2	2.3	0.8	0.6	0.7	0.4	0
18	3.4	2.5	0.8	0.6	0.7	0.4	0
19	3.6	2.7	0.8	0.7	0.7	0.4	0
20	3.8	2.9	0.9	0.7	0.7	0.5	0
21	3.9	3.0	0.9	0.7	0.7	0.5	0
22	4.1	3.1	0.9	0.7	0.8	0.5	0
23	4.3	3.2	1.0	0.8	0.8	0	0
24	4.4	3.4	1.0	0.8	0.8	0	0
25	4.6	3.5	1.1	0.8	0	0	0
26	4.7	3.6	1.2	0.8	0	0	0
27	4.9	3.7	1.4	0.8	0	0	0
28	5.0	3.9	1.5	0.9	0	0	0
29	5.2	4.1	1.6	0.9	0	0	0
30	5.3	4.2	1.7	1.0	0	0	0
31	5.5	4.4	1.8	1.0	0	0	0
32	5.7	4.6	1.9	1.0	0	0	0
33	5.9	4.7	2.0	1.1	0	0	0
34	6.1	4.9	2.1	1.1	0	0	0
35	6.2	5.1	2.2	1.1	0	0	0
36	6.4	5.2	2.3	1.1	0	0	0
37	6.6	5.3	2.4	1.2	0	0	0

*0 indicates no growth

TABLE VI

MEAN DIAMETERS IN CENTIMETERS OF COLONIES GROWN IN VARIOUS CONCENTRATIONS OF METHYLENE BLUE, AT A TEMPERATURE OF APPROXIMATELY 22° C.

Nutrient agar (product of Digestive Ferments Co.) pH 6.8

Days after inoculation	Percentage concentration of dye						
	.00001	.000025	.00005	.000075	.0001	.00025	.0005
1	0.3	0.3	0.3	0.3	0.2	0.4	0.3
2	0.3	0.3	0.3	0.3	0.2	0.4	0.3
3	0.3	0.3	0.3	0.3	0.2	0.4	0.3
4	0.5	0.4	0.3	0.3	0.2	0.4	0.3
5	0.7	0.5	0.3	0.3	0.2	0.4	0.3
6	0.9	0.6	0.4	0.4	0.2	0.4	0.3
7	1.1	0.8	0.5	0.4	0.3	0.4	0.3
8	1.4	1.0	0.5	0.4	0.3	0.5	0.4
9	1.6	1.1	0.6	0.4	0.4	0.5	0.4
10	1.8	1.3	0.7	0.5	0.4	0.5	0.4
11	2.0	1.5	0.9	0.6	0.5	0.5	0.4
12	2.2	1.7	1.0	0.7	0.5	0.5	0.5
13	2.4	1.8	1.1	0.8	0.6	0.5	0.5
14	2.6	2.0	1.2	0.8	0.6	0.5	0.5
15	2.8	2.1	1.3	0.8	0.6	0.5	0.5
16	2.9	2.3	1.5	0.9	0.7	0.6	0.5
17	3.1	2.5	1.6	0.9	0.7	0.6	0.6
18	3.4	2.7	1.7	0.9	0.7	0.6	0.6
19	3.6	2.8	1.8	1.0	0.8	0.6	0.6
20	3.8	3.0	1.9	1.0	0.8	0.6	0.7
21	4.0	3.2	2.0	1.0	0.8	0.7	0.7
22	4.1	3.4	2.1	1.1	0.9	0.7	0.7
23	4.4	3.5	2.2	1.1	0.9	0.7	0.8
24	4.6	3.6	2.4	1.2	1.0	0.7	0.8
25	4.7	3.8	2.4	1.3	1.0	0.7	0.8
26	4.9	4.0	2.6	1.3	1.1	0.8	0.8
27	5.1	4.2	2.7	1.4	1.1	0.8	0.8
28	5.2	4.3	2.9	1.6	1.2	0.8	0.8
29	5.4	4.4	3.0	1.7	1.2	0.9	0.8
30	5.6	4.6	3.2	1.8	1.3	0.9	0.9
31	5.7	4.7	3.3	1.8	1.3	0.9	0.9
32	5.9	4.9	3.5	1.9	1.4	0.9	0.9
33	6.1	5.1	3.6	2.1	1.5	1.0	0.9
34	6.3	5.2	3.8	2.2	1.6	1.0	0.9
35	6.5	5.3	4.0	2.3	1.7	1.1	0.9
36	6.7	5.4	4.1	2.4	1.8	1.1	0.9
37	6.8	5.5	4.2	2.5	1.9	1.2	0.9

TABLE VII

MEAN DIAMETERS IN CENTIMETERS OF COLONIES GROWN IN VARIOUS CONCENTRATIONS OF EOSINE B, AT A TEMPERATURE OF APPROXIMATELY 22° C.

Nutrient agar (product of Digestive Ferments Co.) pH 6.8

Days after inoculation	Percentage concentration of dye						
	.00001	.000025	.00005	.000075	.0001	.00025	.0005
1	0.3	0.2	0.3	0.3	0.5	0.4	0.5
2	0.3	0.2	0.3	0.3	0.5	0.4	0.5
3	0.3	0.2	0.3	0.3	0.5	0.4	0.5
4	0.4	0.4	0.4	0.6	0.7	0.4	0.7
5	0.6	0.5	0.6	0.7	0.9	0.6	0.7
6	0.7	0.6	0.7	0.8	1.0	0.7	0.8
7	0.9	0.9	0.9	1.0	1.2	0.9	1.0
8	1.2	1.2	1.2	1.3	1.4	1.1	1.3
9	1.3	1.3	1.3	1.4	1.6	1.3	1.4
10	1.5	1.5	1.5	1.6	1.8	1.5	1.6
11	1.6	1.7	1.7	1.8	2.0	1.8	1.8
12	1.9	1.9	1.9	2.0	2.2	2.0	2.1
13	2.1	2.1	2.1	2.2	2.4	2.2	2.3
14	2.3	2.3	2.3	2.5	2.6	2.4	2.5
15	2.5	2.5	2.5	2.6	2.9	2.6	2.7
16	2.7	2.7	2.7	2.9	3.1	2.8	2.9
17	2.9	2.9	2.9	3.1	3.3	3.1	3.1
18	3.1	3.1	3.2	3.2	3.5	3.2	3.2
19	3.3	3.3	3.3	3.4	3.6	3.4	3.2
20	3.5	3.4	3.5	3.5	3.8	3.5	3.2
21	3.6	3.6	3.7	3.7	3.9	3.7	0
22	3.8	3.8	3.8	3.9	4.1	3.8	0
23	3.9	4.0	4.0	4.0	4.2	3.9	0
24	4.1	4.2	4.2	4.2	4.3	4.1	0
25	4.2	4.4	4.3	4.3	4.5	4.3	0
26	4.4	4.5	4.5	4.5	4.6	4.5	0
27	4.6	4.7	4.7	4.6	4.8	4.6	0
28	4.7	4.8	4.8	4.8	4.9	4.8	0
29	4.9	5.0	5.0	5.0	5.1	5.0	0
30	5.1	5.2	5.2	5.1	5.2	5.1	0
31	5.3	5.3	5.3	5.3	5.4	5.3	0
32	5.4	5.5	5.5	5.5	5.5	5.5	0
33	5.6	5.7	5.7	5.6	5.7	5.5	0
34	5.7	5.8	5.8	5.8	5.8	0	0
35	5.9	6.0	6.0	5.9	6.0	0	0
36	6.0	6.1	6.1	6.1	6.1	0	0
37	6.2	6.3	6.3	6.2	6.2	0	0

The same microscopical features were also observed in the case of methylene blue, but not quite so outstanding. The rates of growth for both crystal violet and methylene blue, as seen in figs. 11 and 13 respectively, showed a decrease with a more marked sudden drop in the case of crystal violet. These may perhaps be better illustrated in figs. 12 and 14, both being corrected for the inoculum so that the curves represent the total growth at each concentration of the dye for the period denoted. The growth on crystal violet showed a sudden decrease which comes to zero at a concentration of .0005. In the case of methylene blue, however, the decrease was gradual, and the curve may be said to be practically straight.

These results are therefore in accord with the genesistasis beliefs of Churchman or the "bacteriostatic properties," and may be further substantiated by the observation that the inoculum of the crystal violet medium of concentration .0005, which had shown no growth over a period of four months, was transferred to fresh medium and developed into a normal and healthy culture.

A phenomenon which is difficult to explain in the light of this work is the fact that in concentrations of .0001, .00025 and .0005, where there had been very little or no growth, a renewed activity occurred approximately five months after inoculation, in flasks where there had been a rather large amount of agar which had not dried out. A possible explanation may be perhaps that the organism had become accustomed to the dye and, there being enough nutrient in the medium, it began to grow, developing hyphae from the chlamydospores which had been dormant. A microscopic examination of these renewed viability cultures showed many chlamydospores (round) and thick-walled hyphae still present. A chemical interpretation of this phenomenon has as yet not been postulated and will perhaps require much more work and a better understanding of the reaction.

In the case of eosine B, growth was normal on all concentrations up to the nineteenth day, as seen in table VII, when growth actually ceased in the medium of the highest concentration of the dye (.0005). This was followed by a cessation in growth on the thirty-fourth day for the concentration .00025. This phenomenon has been noticed and reported in previous papers by the

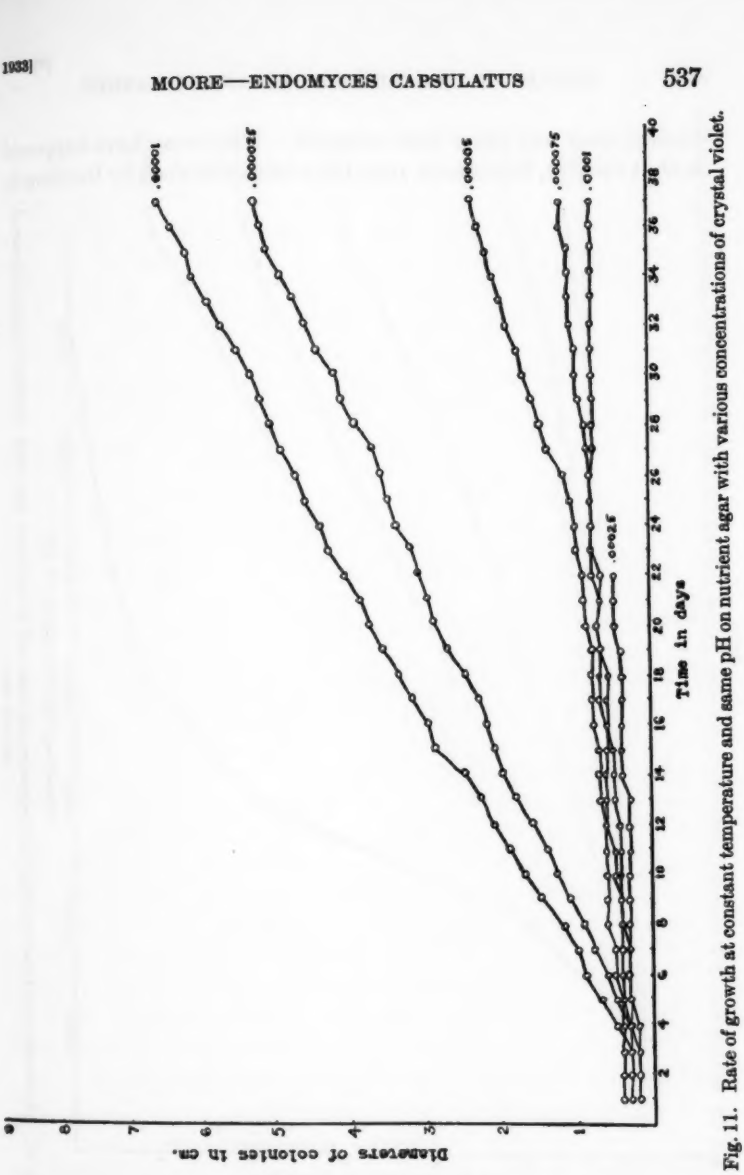


Fig. 11. Rate of growth at constant temperature and same pH on nutrient agar with various concentrations of crystal violet.

author for eosine-methylene-blue agar where the mycelium had taken on a pink to blue color and ceased growing. These cultures when transplanted to fresh agar grew just as luxuriantly as

though they had never been affected. What may have happened is that the dye, being acid, may have been absorbed by the fungus,

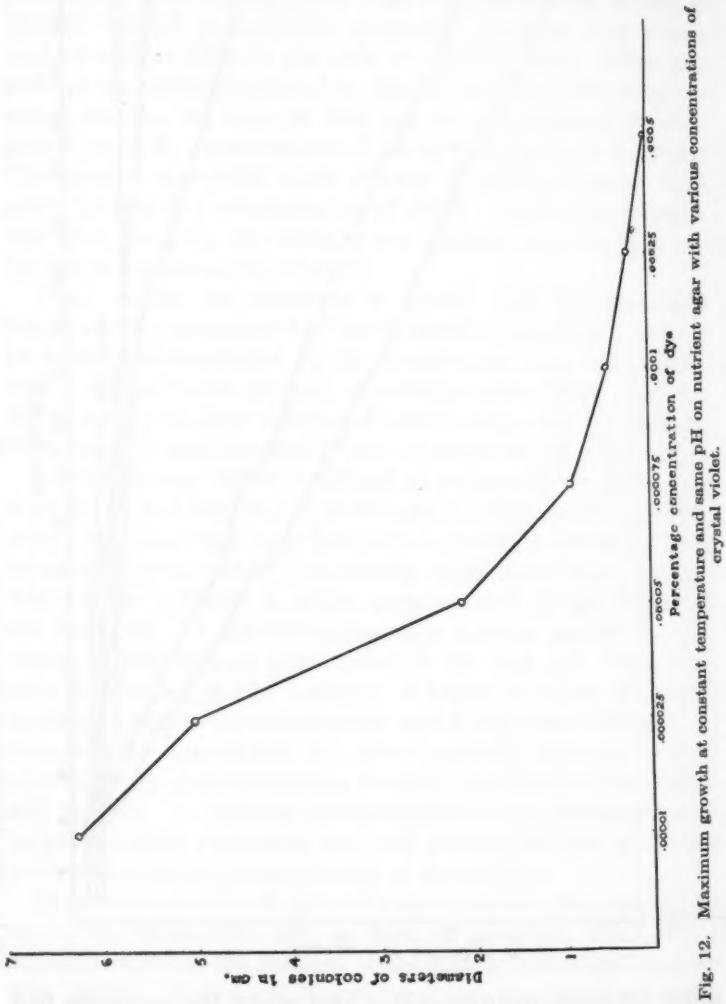


Fig. 12. Maximum growth at constant temperature and same pH on nutrient agar with various concentrations of crystal violet.

as evidenced by the color of the mycelium, and was strong enough to counteract the metabolic activities of the cell. It would seem

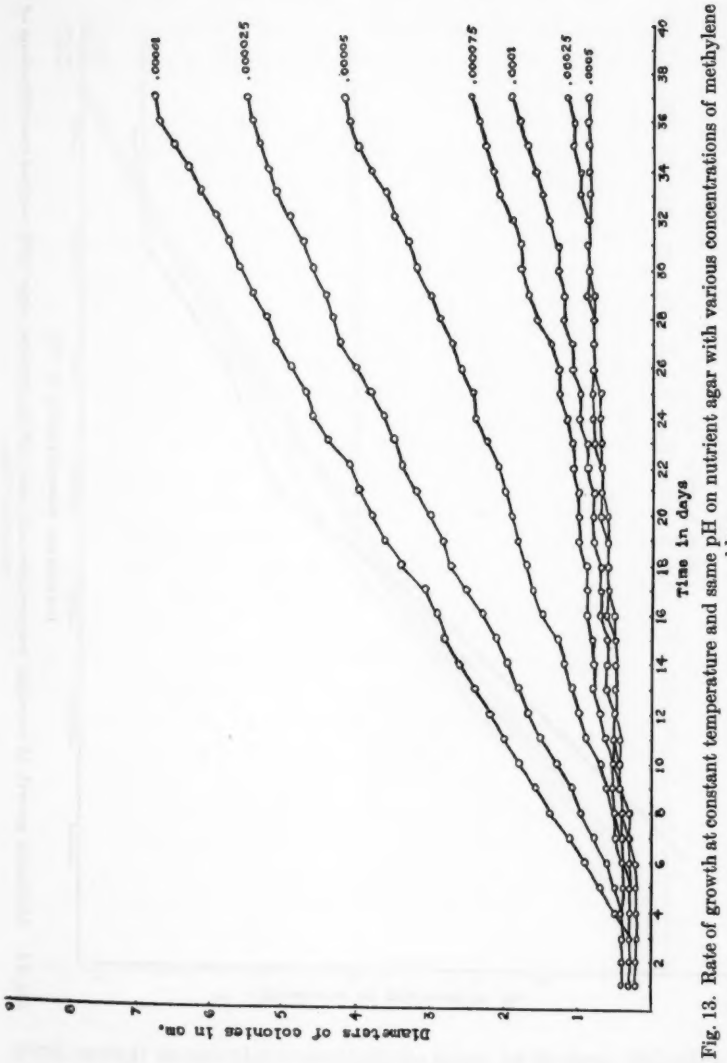


Fig. 13. Rate of growth at constant temperature and same pH on nutrient agar with various concentrations of methylene blue.

from a study of the pH of the medium, which changes from a slightly acid to alkaline in normal growth, that this could be detected. However, the medium was already covered with

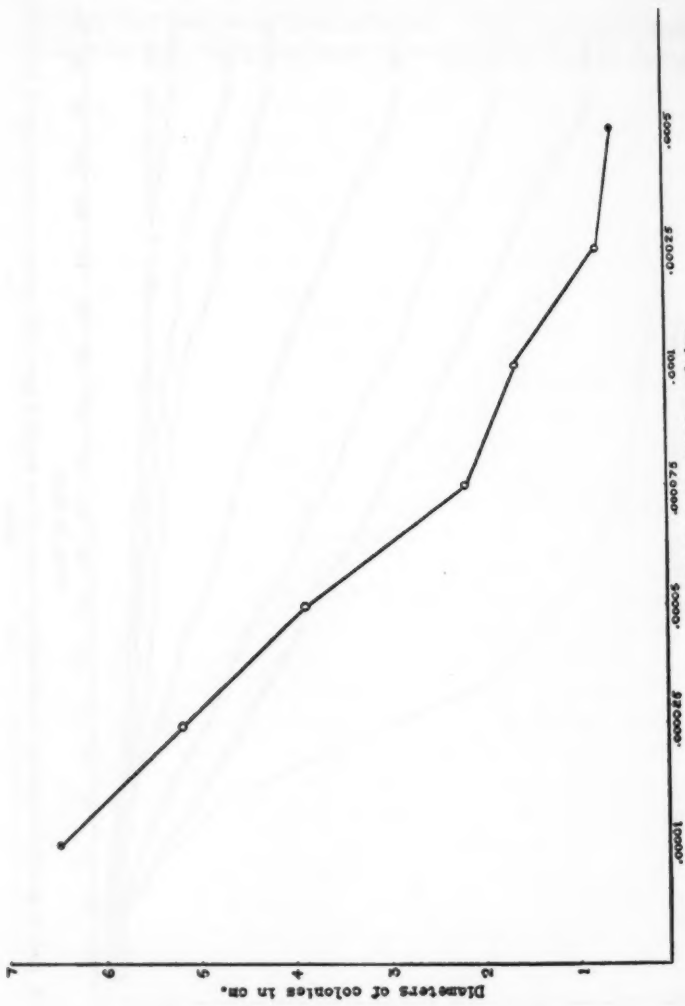


Fig. 14. Maximum growth at constant temperature and same pH on nutrient agar with various concentrations of methylene blue.

sufficient growth to cause an alkaline condition, so that no direct evidence could be obtained from that direction. There are no definite facts to uphold this belief, and the statement should be considered more as a conjecture for the present. There have

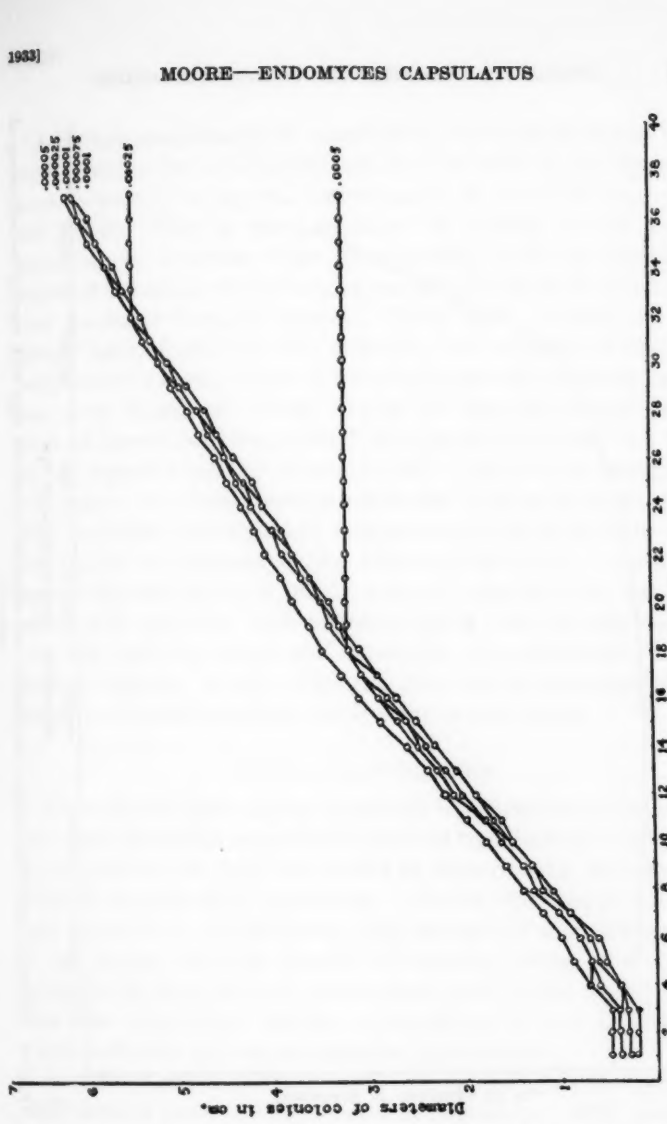


Fig. 15. Rate of growth at constant temperature and same pH on nutrient agar with various concentrations of eosine B.

been many theories proposed, but none have had any final acceptance.

The action of eosine B on the organism is illustrated in figs. 15–16. The former shows the rate of growth of the cultures on

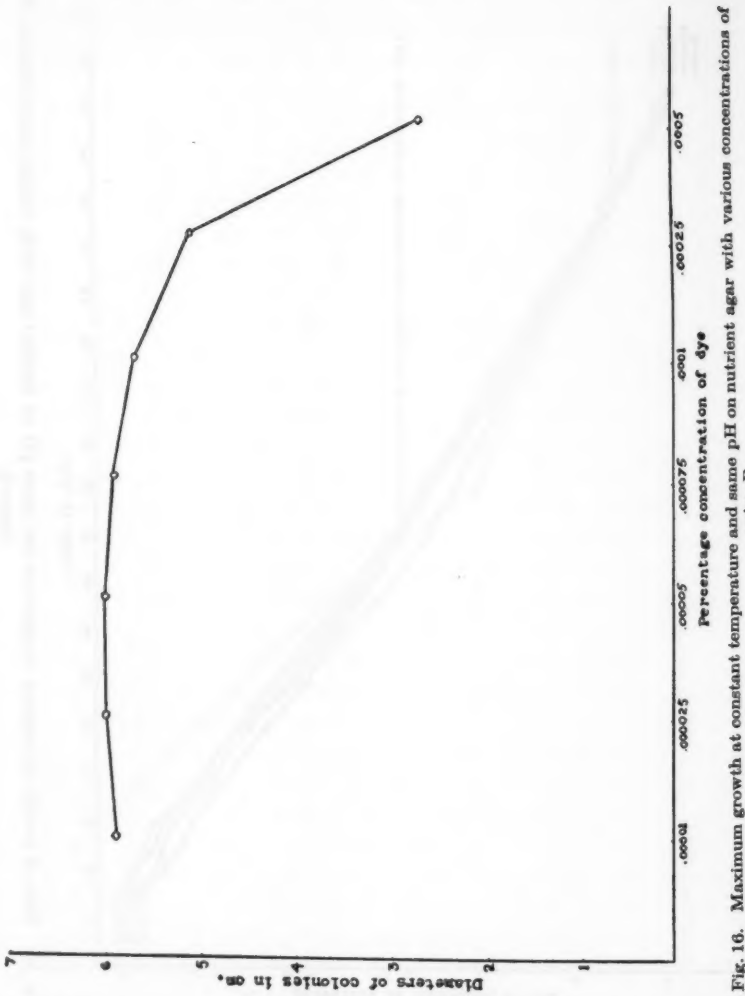


Fig. 16. Maximum growth at constant temperature and same pH on nutrient agar with various concentrations of eosine B.

various concentrations of dye. Growth on the concentration .0005 was inhibited on the nineteenth day, and that on the concentration .00025, on the thirty-fourth day. Fig. 16 represents the maximal points of growth corrected for the inoculum. The inhibition of growth is clearly distinguishable here.

A further phenomenon is noted here, not because it was absolutely specific for this experiment but because it had appeared most frequently on crystal violet media of concentrations .0001 and .00025. This is the formation of crystals in the media, appearing to emanate from the growing zone or peripheral region of the colony and found in conditions which have not been very favorable towards growth. They have occurred on beef extract agar of pH 7.2, 7.0, and 6.8. No crystals of this sort were found on media where there was no growth or where growth was very luxuriant. They appear to resemble very closely those of ammonium magnesium phosphate ($NH_4MgPO_4.6\ H_2O$) as was reported also by Scudder ('26). They were insoluble in cold water, completely and immediately soluble in hydrochloric acid, and were reprecipitated with sodium hydroxide, tests which are applied to crystals of the above composition. They were apparently the result of fungal activity, and Scudder has associated this condition with bacteria which "do not ferment the ordinary carbohydrates and intensify the alkalinity of the medium rapidly," a fact which has been clearly demonstrated in the carbohydrate reactions carried out in this paper.

Animal Inoculations

From time to time papers appear in the literature pointing out that some particular organism which had been kept in culture over a long period and then inoculated in experimental animals, had retained its pathogenic properties. On the other hand, it is quite well known that this property may be lost on subculturing. It is also known that by growth on certain media at a specific temperature, toxicity may be retained over a long time. It has also been found that certain microorganisms may be made to regain lost toxic powers by repeated inoculations.

In order to determine whether *E. capsulatus* had undergone any such changes since it was isolated in September, 1928, and kept in culture at different temperatures, on different media, and at various hydrogen-ion concentrations, several animals were inoculated with a twelve-day-old suspension of the organism in 10-cc. physiological saline solution. The culture was stirred up thoroughly with a sterile needle to eliminate the clumps.

The animals and inoculations were as follows:

Rabbits: Two females—intracerebral (0.2 cc.) and intravenous (0.5 cc.). Two males—subcutaneous (2 cc.) and intratesticular (1 cc.).

Guinea-pigs: Two, one male and one female—intracerebral (0.2 cc.). Two males—subcutaneous (1.5 cc.) and intratesticular (1 cc.).

Mice: Two, one male and one female—intracerebral (0.2 cc.). One male—subcutaneous (1 cc.) and intratesticular (0.5 cc.).

The results of these inoculations were that one of the rabbits receiving a subcutaneous and intratesticular inoculation died twenty-three days later. Autopsy revealed no infection or lesion, except an intestinal obstruction. Three mice, healthy, with no loss of weight or appetite, were killed fifty days after inoculation. The organs were completely negative. Cultures of the heart's blood also negative. The rabbits and guinea-pig were healthy, with no loss of weight, three months after inoculation. The remaining mouse, with an intracerebral injection, died sixty-two days after inoculation as a result of pneumonia. Autopsy showed no complication of the other organs. Cultures of infected lung tissue and brain negative for *E. capsulatus*, the lung in a hemorrhagic condition.

It may be concluded, therefore, that the organism had lost its power to infect after four years' growth on artificial substrates. That it failed to produce a toxic action in an animal may be attributed to the changed environment and habitat of the fungus. It has been pointed out previously that the optimum temperature has been reduced from 37.5° C. to approximately 25° C. In addition, experimental data has shown that growth at the former temperature was very slow and slight, with complete cessation and death after a short time. It is very possible that the organism was killed here, as in the *in vitro* experiments, as a result of the body temperature of the experimental animals. A further point of interest is the condition of aerobiosis as found here. When freshly isolated, the yeast-cells of *E. capsulatus* or *E. dermatitidis* are able to live in a condition of facultative aerobiosis, that is, with or without oxygen, favoring the presence of air. With continued cultivation on artificial media, the organism

adapts itself to a saprophytic form of life and as a result becomes a strict aerobe, requiring oxygen for its existence. This change to strict aerobiosis is an important factor, since, by inoculation in the animal body, the organism is forced into an aerobic condition which it cannot endure and, as probably happened here, it is killed for lack of oxygen.

There are probably several other factors which may account for this failure of the organism to cause infection, but what has been said above accounts for the chief difficulties.

Summary and Conclusions

1. A review of the case histories from which the organisms *Endomyces capsulatus*, *E. capsulatus* var. *isabellinus*, *E. dermatitidis*, and the Austrian fungus were isolated are given.

2. A study of the organism shows that it has two life cycles: one in the parasitized host as a budding yeast-cell with the chromatin material spread throughout the cell; and the other as a perfect Ascomycete, reproducing by the development of 8-spored asci resulting from a sexual act which may be either hetero- or isogamy or have become reduced to parthenogamy.

3. No definite form of mitosis is demonstrable; instead conditions similar to those described as amitosis are recorded.

4. Cellular contents, as volutin, glycogen, vacuoles, chondriosomes, and fat or lipoidal substances, are demonstrated in *E. capsulatus* by various methods.

5. The cultural work is described in detail and includes descriptions of the organism on 29 different media, showing that hydrogen-ion concentration may influence growth as expressed by thin hyphae and numerous conidia on acid media and thick-walled, shorter cells on alkaline media.

6. After four years of subculturing an optimum temperature of 25° C. is determined for the organism, with no growth occurring above 37° C. or below 8.0° C.

7. An optimum pH of 7.4 is determined for *E. capsulatus*, with no growth occurring at a pH greater than 9.3 or less than 3.3.

8. The carbohydrates used in this work require a variation in time for the production of alkalinity. No acid or gas is produced. Hydrogen-ion measurements show a decrease from

the initial pH of 6.8 to an end point of approximately 8.1, which is accounted for by the production of ammonia and probably hexone bases.

9. Under proper conditions, indol and skatol reactions are present.

10. In the yeast form *E. capsulatus* may show facultative anaerobiosis, but growth on an artificial substrate converts the organism into a strict aerobe.

11. Nitrate reduction and hydrogen sulphide production yield indefinite results.

12. Gelatine is slowly liquefied after 30 days.

13. Light, either white or red, has no effect on the organism as compared with that of dark or diffuse daylight and total darkness.

14. Several dyes, orange G, Sudan III, alizarin, neutral red, safranine A, light green SF, basic fuchsin, aniline blue, phenol red, and haematoxylin, in concentrations of .00001, .000025, .00005, .000075, .0001, .00025, .0005, when incorporated in an agar medium have no effect on growth. Crystal violet and methylene blue in the same concentrations show a gradual decrease in growth, with complete cessation at a concentration of .0005 for crystal violet. Crystal violet showed a reduction in the number of nuclear divisions, sexual fusions, and production of asci, with an accompanying enlargement of the cells, development of thick walls, secretion of granular material in the cytoplasm, conditions associated with the formation of chlamydospores under unfavorable conditions. Eosine B has an effect on growth in high concentrations.

15. Animal inoculations for pathogenicity were negative after subculturing for a period of approximately four years.

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Bibliography

Atkinson, G. F. ('15). Phylogeny and relationships in the Ascomycetes. Mo. Bot. Gard., Ann. **2**: 315–376. 1915.

Bagchee, K. ('25). Cytology of the Ascomycetes. Pustularia bolarioides. Ann. Bot. **39**: 217–266. 1925.

Blackman, V. H., and H. C. I. Fraser ('05). Fertilization in Sphaerotheca. *Ibid.* **19**: 567–569. 1905.

Brooks, F. T. ('10). The development of Gnomonia erythrostoma Pers. *Ibid.* **24**: 585–605. 1910.

Brown, W. H. ('10). The development of the ascocarp of Leotia. Bot. Gaz. **50**: 443–459. 1910.

———, ('11). The development of the ascocarp of Lachnea scutellata. *Ibid.* **52**: 275–305. 1911.

Carruthers, D. ('11). Contributions to the cytology of Helvella crispa. Ann. Bot. **25**: 243–252. 1911.

Chamberlain, C. J. ('32). Methods in plant histology. 5th rev. ed. 416 pp. Univ. of Chicago Press. 1932.

Chiari, H. ('30). Zur Pathologie und Histologie der generalisierten Torulose (Blastomykose). Arch. f. Derm. u. Syphil. **162**: 422–441. 1930.

Churchman, J. W. ('12). The selective bactericidal action of gentian violet. Jour. Exp. Med. **16**: 221–248. 1912.

———, ('20). The cause of the parallelism between the Gram reaction and the gentian violet reaction. Soc. Exp. Biol. & Med., Proc. **18**: 17–18. 1920.

———, ('20a). The isolation of gentian positive individuals from a gentian negative organism (B. coli). *Ibid.* 19. 1920.

———, ('20b). Relation of the gentian violet reaction to dilution of implanted suspension. *Ibid.* 20. 1920.

———, ('20c). The effect of repeated inoculations of gentian violet agar with gentian positive organisms. *Ibid.* 20–21. 1920.

———, ('20d). The selective action of gentian violet in relation to chemotherapy. *Ibid.* 21–22. 1920.

———, ('20e). The communal activity of bacteria. *Ibid.* 22–23. 1920.

———, ('21). Further studies on the behavior of bacteria toward gentian violet. Jour. Exp. Med. **33**: 569–582. 1921.

———, and M. C. Kahn ('21). Communal activity of bacteria. *Ibid.* 583–592. 1921.

Clark, E. B. ('27). Effect of gentian violet and mercurochrome on Endomyces albicans, with special reference to the mycelial form. Jour. Infect. Dis. **40**: 423–424. 1927.

Claussen, P. ('06). Über neuere Arbeiten zur Entwicklungsgeschichte der Ascomyceten. Ber. d. Deut. Bot. Ges. **24**: (11)–(38). 1906.

Concetti, L. ('00). Biologie et pathogénie du muguet. Arch. Méd. Enf. 3: 479, 517–541, 590–605. 1900.

Cowdry, N. H. ('17). A comparison of mitochondria in plant and animal cells. Biol. Bull. 33: 196–228. 1917.

Dangeard, P. A. ('93). Sur la structure histologique des levures et leur développement. Compt. Rend. Acad. Sci. 117: 68–70. 1893.

———, ('94). Recherches sur la reproduction sexuelle des champignons. Le Botaniste 3: 221–281. 1894.

———, ('94a). La structure des levures et leur développement. *Ibid.* 282–286. 1894.

———, ('94b). La reproduction sexuelle des Ascomycètes. *Ibid.* 4: 21–58. 1894.

———, ('97). Second mémoire sur la reproduction sexuelle des Ascomycètes. *Ibid.* 5: 245–284. 1897.

———, ('04–'06). Recherches sur le développement du périthèce chez les Ascomycètes. *Ibid.* 9: 59–303. 1904–1906.

———, ('07). *Ibid.* II. L'origine du périthèce chez les Ascomycètes. *Ibid.* 10: 1–385. 1907.

Devereux, E. D., and F. W. Tanner ('27). Observations on the growth of yeasts in pure nutrient solutions. Jour. Bact. 14: 317–333. 1927.

Faber, H. K., and L. B. Dickey ('25). The treatment of thrush with gentian violet. Am. Med. Assoc., Jour. 85: 900–901. 1925.

Farley, D. L. ('20). The use of gentian violet as a restrainer in the isolation of pathogenic moulds. Arch. Derm. & Syphil. 2: 459–465. 1920.

———, ('20a). The cultivation of Epidermophyton inguinale. *Ibid.* 466–469. 1920.

Faull, J. H. ('05). Development of the ascus and spore formation in Ascomycetes. Boston Soc. Nat. Hist., Proc. 32: 77–114. 1905.

Fellers, C. R., and R. W. Clough ('25). Indol and skatol determination in bacterial cultures. Jour. Bact. 10: 105–133. 1925.

Fineman, B. C. ('21). A study of the thrush parasite. Jour. Infect. Dis. 28: 185–200. 1921.

Fraser, H. C. I., and J. W. St. John Brooks ('09). Further studies on the cytology of the ascus. Ann. Bot. 23: 537–549. 1909.

———, and E. J. Welsford ('08). Further contributions to the cytology of the Ascomycetes. *Ibid.* 22: 465–477. 1908.

Fuhrmann, F. ('06). Die Kernteilung von Saccharomyces ellipsoideus I. Hansen bei der Sprossbildung. Centralbl. f. Bakt. II, 15: 769–777. 1906.

Gaŭmann, E. A., and C. W. Dodge ('28). Comparative morphology of fungi. 701 pp. McGraw Hill Book Co. New York. 1928.

Gnezda, J. ('99). Sur les réactions nouvelles des indoliques et des corps albuminoides. Compt. Rend. Acad. Sci. 128: 1584–1587. 1899.

Goré, S. N. ('21). The cotton wool plug test for indole. Indian Jour. Med. Res. 8: 505–507. 1921.

Greenbaum, S. S., and J. V. Klauder ('22). Yeast infections of the skin: Report of cases and of studies on the cutaneous yeasts. Arch. Derm. & Syphil. 5: 332–344. 1922.

Guilliermond, A. ('03). Recherches cytologiques sur les levures. Rev. Gén. Bot. 15: 49–66, 104–124, 166–185. 1903.

———, ('03a). Contribution à l'étude de l'épiplasme des Ascomycètes et recherches sur les corpuscules métachromatiques des champignons. Ann. Myc. **1**: 201–215. 1903.
———, ('04). Sur le noyau de la levure. *Ibid.* **2**: 184–189. 1904.
———, ('04a). Contribution à l'étude de la formation des asques et de l'épiplasme des Ascomycètes. Rev. Gén. Bot. **16**: 49–65. 1904.
———, ('04b). Recherches sur la karyokinèse chez les Ascomycètes. *Ibid.* 129–143. 1904.
———, ('05). Recherches sur la germination des spores et la conjugaison chez les levures. *Ibid.* **17**: 337–376. 1905.
———, ('05a). La morphologie et la cytologie des levures. Inst. Pasteur, Bull. **3**: 177–184, 225–235. 1905.
———, ('05b). Remarques sur la karyokinèse des Ascomycètes. Ann. Myc. **3**: 343–361. 1905.
———, ('07). À propos de l'origine des levures. *Ibid.* **5**: 49–69. 1907.
———, ('07a). La cytologie des bactéries. Inst. Pasteur, Bull. **5**: 273–283. 1907.
———, ('08). Contribution à l'étude cytologique des bacilles endosporés. Arch. f. Protistk. **12**: 9–43. 1908.
———, ('08a). La question de la sexualité chez les Ascomycètes. Rev. Gén. Bot. **20**: 32–39, 85–89, 111–120, 178–182, 298–305, 332–344, 364–378. 1908.
———, ('09). Sur la phylogenèse des levures. Compt. Rend. Soc. Biol. **66**: 998–1000. 1909.
———, ('09a). Observations sur la cytologie d'un bacille. *Ibid.* **67**: 102–103. 1909.
———, ('09b). Recherches cytologiques et taxonomiques sur les Endomycétées. Rev. Gén. Bot. **21**: 353–391, 401–419. 1909.
———, ('10). La sexualité chez les champignons. Bull. Sci. France et Belg. **44**: 109–196. 1910.
———, ('10a). Remarques critiques sur différentes publications sur la cytologie des levures et quelques observations sur la structure de ces champignons. Centralbl. f. Bakt. II, **26**: 577–589. 1910.
———, ('10b). Remarques sur le développement de l'Endomyces fibuliger (Lindner). Compt. Rend. Soc. Biol. **68**: 318–320. 1910.
———, ('11). Apercu sur l'évolution nucléaire des Ascomycètes. Rev. Gén. Bot. **23**: 89–120. 1911.
———, ('11a). Sur un exemple de copulation hétérogamique observé chez une levure. Compt. Rend. Soc. Biol. **70**: 442–444. 1911.
———, ('11b). Sur les mitochondries des cellules végétales. *Ibid.* **153**: 199–201. 1911.
———, ('12). Nouvelles observations sur la sexualité des levures. Arch. f. Protistk. **28**: 52–77. 1912.
———, ('13). Les progrès de la cytologie des champignons. Prog. Rei Bot. **4**: 389–542. 1913.
———, ('14). Monographie des levures rapportées d'Afrique occidentale. Ann. Sci. Nat. Bot. **19**: 1–32. 1914.
———, ('17). Sur la division nucléaire des levures. Ann. Inst. Pasteur **31**: 107–113. 1917.
———, ('19). Sur une nouvelle levure à copulation hétérogamique. Compt. Rend. Soc. Biol. Paris **82**: 466–470. 1919.

————, ('20). Zygosaccharomyces Pastori, nouvelle espèce de levure à copulation hétérogamique. Soc. Myc. France, Bull. **36**: 203–211. 1920.

————, et G. Péju ('19). Sur un nouveau champignon présentant des caractères intermédiaires entre les levures et les Endomyces. Compt. Rend. Soc. Biol. **82**: 1343–1346. 1919.

————, ————, ('20). Une nouvelle espèce de levure du genre Debaryomyces, D. Klöckeri, n. sp. Soc. Myc. France, Bull. **36**: 164–171. 1920.

————, ————, ('21). Une nouvelle espèce de levure du genre Debaryomyces, D. Nadsonii, n. sp. *Ibid.* **37**: 35–38. 1921.

————, G. Mangenot, et L. Plantefol ('33). Traité de cytologie végétale. 1195 pp. Librairie e Le François. Paris, 1933.

Hansen, E. ('04). Grundlinien zur Systematik der Saccharomyceten. Centralbl. f. Bakt. **12**: 529–538. 1904.

Harper, R. A. ('96). Über das Verhalten der Kerne bei der Fruchtentwickelung einiger Ascomyceten. Jahrb. f. wiss. Bot. **29**: 655–685. 1896.

————, ('97). Kernteilung und freie Zellbindung im Ascus. *Ibid.* **30**: 249–284. 1897.

————, ('99). Cell-division in sporangia and asci. Ann. Bot. **13**: 467–525. 1899.

————, ('00). Sexual reproduction in Pyronema confluens and the morphology of the ascocarp. *Ibid.* **14**: 321–400. 1900.

Holman, W. L., and F. L. Gonzales ('23). A test for indol based on the oxalic acid reaction of Gnezda. Jour. Bact. **8**: 577–583. 1923.

Janssens, A. ('02). À propos du noyau de la levure. La Cellule **20**: 335–349. 1902.

————, et A. Leblanc ('98). Recherches cytologiques sur la cellule de levure. *Ibid.* **14**: 201–243. 1898.

Kater, J. McA. ('27). Cytology of Saccharomyces cerviciae, with especial reference to nuclear division. Biol. Bull. **52**: 436–448. 1927.

————, ('28). Note on the structure of a Monilia isolated from a case of psoriasis. Univ. Calif. Publ. Bot. **14**: 301–306. 1928.

Kohl, F. G. ('07). Über das Glykogen und einige Erscheinungen bei der Sporulation der Hefe. Ber. d. Deut. Bot. Ges. **25**: 74–85. 1907.

Koser, S. A., and R. H. Galt ('26). The oxalic acid test for indol. Jour. Bact. **11**: 293–303. 1926.

Krumwiede, C., und J. S. Pratt ('13). Dahlia-Agar als Unterschiedsungmittel zwischen Cholera und anderen Vibrionen. Centralbl. f. Bakt. Orig. **68**: 562–566. 1913.

Kulp, W. L., and L. F. Rettger ('24). Comparative study of Lactobacillus acidophilus and Lactobacillus bulgaricus. Jour. Bact. **9**: 357–394. 1924.

Lewis, M. R. ('30). Effect of dyes on the virus of chicken tumor. Amer. Jour. Hyg. **12**: 288–296. 1930.

Maire, R. ('04). Sur les divisions nucléaires dans l'asque de la morille et de quelques autres Ascomycètes. Compt. Rend. Soc. Biol. **56**: 822–824. 1904.

————, ('05). Recherches cytologiques sur quelques Ascomycètes. Ann. Myc. **3**: 123–154. 1905.

Mallinckrodt-Haupt, A. St. von ('32). Der Wert der pH-Messung bei Pilzkulturen. Zentralbl. f. Bakt. Orig. **125**: 368–374. 1932.

Malone, R. H., and S. N. Goré ('21). The relation of indole in bacterial culture. Indian Jour. Med. Res. **8**: 490–504. 1921.

Mangenot, G. ('19). Sur la formation des asques chez Endomyces Lindneri (Saïto). Compt. Rend. Soc. Biol. **82**: 477–479. 1919.

Marantonio, R. ('93). Contributo alla biologia del fungo del mughetto. Ann. Ist. Ig. Roma **3**: 199–223. 1893.

Meyer, R. ('28). Über den Einfluss der Temperatur auf den Wachstumablauf bei Pilzen. Biochem. Zeitschr. **198**: 463–477. 1928.

Milochevitch, S. ('29). Das Wachstum und die Wachstumformen des Soorpilzes auf verschiedenen Nährboden besonders auf solchen die mit Extrakten aus Körperorgane Hergestellt wurden. Zentralbl. f. Bakt. Orig. **114**: 174–186. 1929.

Moore, M. ('32). Coccidioidal granuloma: A classification of the causative agent, Coccidioides immitis. Mo. Bot. Gard., Ann. **19**: 397–428. 1932.

———, ('33). Blastomycosis: Report of a case with a study of an etiologic factor and a classification of the organism. *Ibid.* **20**: 79–117. 1933.

[———], MacBryde, C. M., and E. J. Thompson ('33). Meningitis and dermatitis caused by a new variety of blastomycete (endomycete). Arch. of Derm. & Syphil. **27**: 49–69. 1933.

Morelli, G. ('09). Ueber ein neues Verfahren zum Nachweis von Indol auf Nährsubstraten. Centralbl. f. Bakt. Orig. **50**: 413–415. 1909.

Ota, M. ('24). Beiträge zur Morphologie, Biologie und Systematik der pathogenen, asporogenen Sprosspilze. Dermat. Wochenschr. **78**: 216–237, 260–265. 1924.

Petroff, S. A. ('15). A new and rapid method for the isolation and cultivation of tubercle bacilli directly from sputum and feces. Jour. Exp. Med. **21**: 38–42. 1915.

Pittaluga, G. ('08). Sobre un nuevo metodo para la investigacion del indol en la practica bacteriologica. [Abs. in Bull. l'Inst. Pasteur **6**: 578–579. 1908.]

Rewbridge, A. G., C. W. Dodge, and T. T. Ayers ('29). A case of meningitis due to Endomyces capsulatus (new species). Amer. Jour. Path. **5**: 349–364. 1929.

Salkowski, E. ('83). Zur Kenntniss der Eiweissfäulnis 1: Ueber die Bildung des Indols und Skatols, nach gemeinschaftlich mit H. Salkowski in münster i/w angestellten Versuchen. Zeitschr. Physiol. Chem. **8**: 416–466. 1883.

Sanderson, E. S., and D. C. Smith ('27). The effect of gentian violet on the organism of blastomycotic infection. Arch. Derm. & Syphil. **16**: 153–155. 1927.

Sasaki, T. ('10). Über eine neue empfindliche Skatolreaktion. Biochem. Zeitschr. **23**: 402–403. 1910.

Scudder, S. A. ('26). Crystal formation in bacterial cultures. Jour. Bact. **11**: 90–91. 1926.

Shrewsbury, J. F. D. ('30). The genus Willia. Jour. Path. & Bact. **33**: 393–416. 1930.

Smith, T. ('99). The relation of dextrose to the production of toxin in bouillon cultures of the diphtheria bacillus. Jour. Exp. Med. **4**: 373–397. 1899.

Spring, D. ('29). Comparison of seven strains of organisms causing blastomycoses in man. Jour. Infect. Dis. **44**: 169–185. 1929.

Stearn, E. W., and A. E. Stearn ('23). The mechanical behavior of dyes especially gentian violet, in bacteriological media. Jour. Bact. **8**: 567–572. 1923.

———, ———,('29). Comparative inhibiting effect of gentian violet and mercurochrome on the growth of certain fungi. Jour. Lab. & Clin. Med. **14**: 1057–1060. 1929.

Steensma, F. A. ('06). Über Farbenreaktionen der Eiweisskörper, des Indols und

des Skatols mit aromatischen Aldehyden und Nitriten. Zeitschr. physiol. Chem. **47**: 25–27. 1906.

———, ('06a). Über den Nachweis von Indol und die Bildung von Indol vortäuschenden Stoffen in Bakterienkulturen. Centralbl. f. Bakt. Orig. **41**: 295–298. 1906.

Stendel, H., und E. Peiser ('19). Über die Hefenucleinsäure. Zeitschr. physiol. Chem. **108**: 42–49. 1919.

Swellengrebel, M. ('05). Sur la division nucléaire de la levure pressée. Ann. Inst. Pasteur **19**: 503–515. 1905.

Talice, R. ('30). Sur la filamentisation des Monilia. Ann. Parasitol. Hum. Comp. **8**: 394–410. 1930.

Thomas, P. ('21). Recherches sur les protéiques de la levure. Ann. Inst. Pasteur **35**: 43–95. 1921.

Urbach, E., und F. Zach ('30). Generalisierte Torulose (europäische Blastomykose). Eine klinisch-botanische Studie. Arch. f. Derm. u. Syphil. **162**: 401–421. 1930.

Wager, H. ('98). The nucleus of the yeast-plant. Ann. Bot. **12**: 499–544. 1898.

———, and A. Peniston ('10). Cytological observations on the yeast-plant. *Ibid.* **24**: 45–84. 1910.

Watanabe, J. ('32). The action of sugar on amino acid. I. The reaction in alkaline medium. Jour. Biochem. **16**: 163–190. 1932.

Wetmore, R. H. ('32). The use of celloidin in botanical technic. Stain Technol. **7**: 37–62. 1932.

Zikes, H. ('22). Beitrag zum Volutinvorkommen in Pilzen. Centralbl. f. Bakt. II, **57**: 21–45. 1922.

Zinsser, H. ('29). A textbook of bacteriology. 1053 pp. 6th ed. D. Appleton and Co., New York. 1929.

Zipfel, H. ('12). Zur Kenntnis der Indolreaktion. Centralbl. f. Bakt. Orig. **64**: 65–80. 1912.

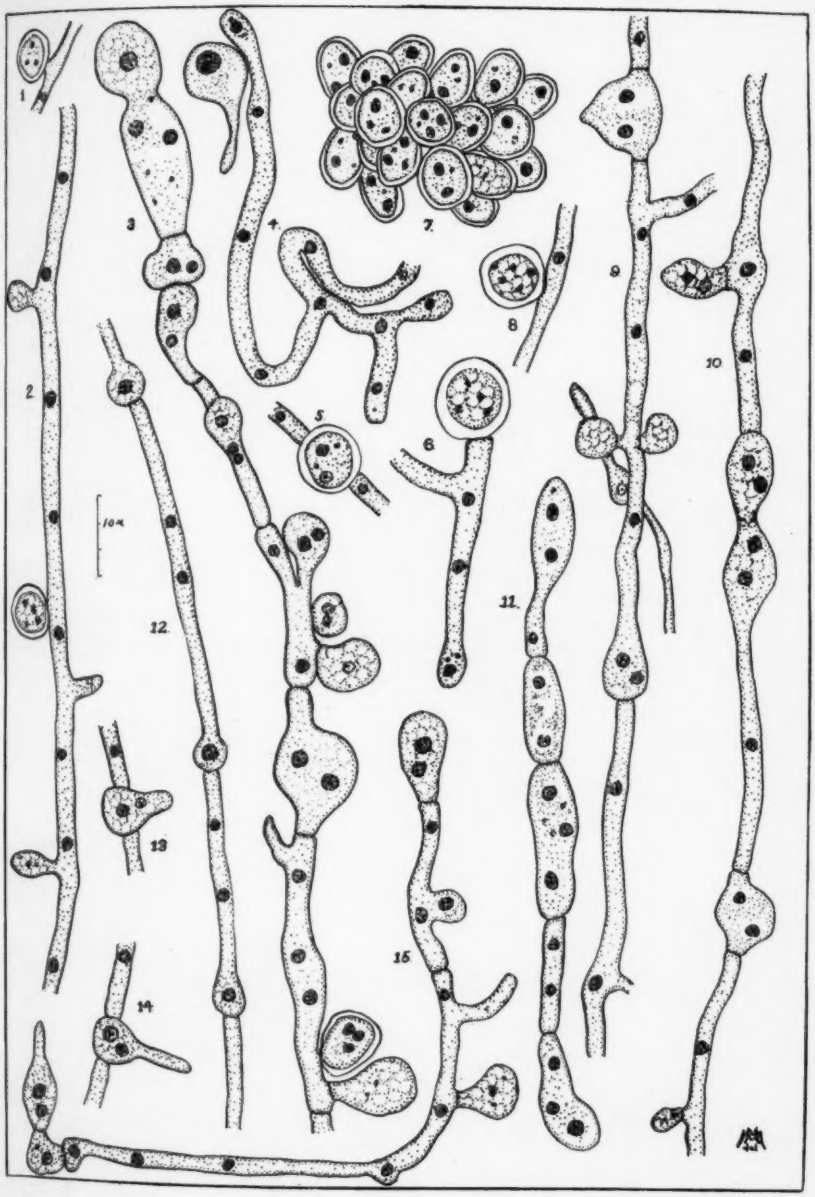
Zoller, H. F. ('20). Influence of hydrogen ion concentration upon the volatility of indole from aqueous solution. Jour. Biol. Chem. **41**: 37–44. 1920.

Explanation of Plate

PLATE 16

All figures drawn at a magnification of × 1440 with the aid of a camera lucida.

Fig. 1. Thick-walled cell on glycerine agar.
Fig. 2. Young mycelium.
Fig. 3. Mature mycelium with conidia, terminal hypnospores, and oidia.
Fig. 4. Mycelium on blood agar.
Fig. 5. Intercalary chlamydospore.
Fig. 6. Terminal hypnospore.
Fig. 7. Group of thick-walled cells on bacto-peptone plus glycerine.
Fig. 8. Thick-walled cell on corn-meal agar.
Fig. 9. Mycelium with racquet hyphae, conidia, and intercalary chlamydospore.
Fig. 10. Mycelium grown on calcium carbonate medium.
Fig. 11. Mycelium on glycerine agar.
Fig. 12. Racquet mycelium on Sabouraud's agar.
Fig. 13. Endo-chlamydospore.
Fig. 14. Growth of fig. 13.
Fig. 15. Mycelium with conidia, young developing hyphal branch, and hypnospores on beef extract agar.



MOORE—ENDOMYCES CAPSULATUS

AVON

EXPLANATION OF PLATE

PLATE 17

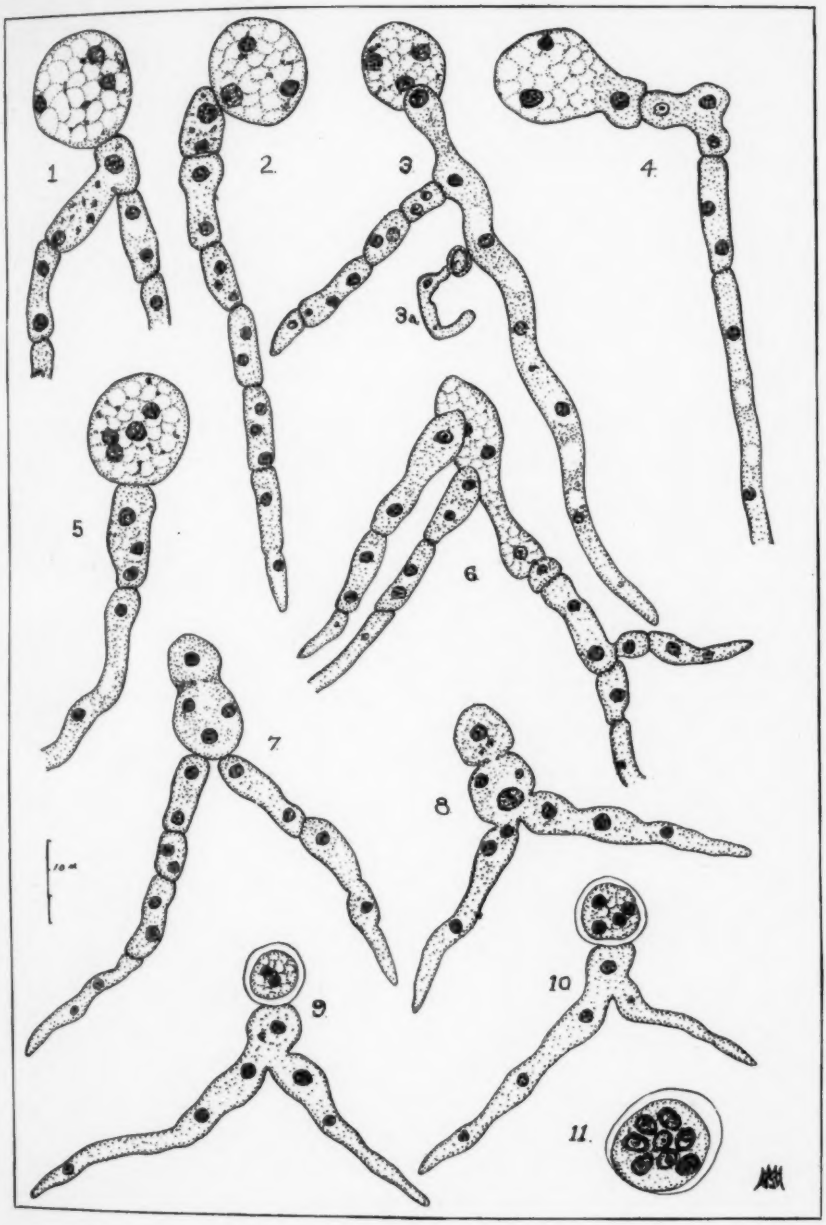
All drawings made from a hanging-drop culture of bacto-peptone plus glycerine, with the aid of a camera lucida at × 1440.

Figs. 1–5. Chlamydospores and young hyphae.

Fig. 3a. Young germinating spore.

Fig. 6. Group of germinating spores.

Figs. 7–11. Developmental series observed over a period of 3 days with the final formation of an ascus with 8 spores (fig. 11). Condition analogous to the Stoppel form of fertilization.



MOORE—ENDOMYCES CAPSULATUS

Explanation of Plate

PLATE 18

All figures drawn with the aid of a camera lucida at a magnification of × 1440.

Fig. 1. Hypha on malt extract agar showing chlamydospores.

Fig. 2. Terminal chlamydospore or ascogonial cell.

Fig. 3. Hyphal termination.

Figs. 4–5. Yeast-like cells with thick walls, grown on beef extract plus blood serum agar.

Fig. 6. Hypha with pyriform conidia, lateral chlamydospores on wort agar.

Fig. 7. Hyphae showing an abundance of conidia on Raulin's solution agar.

Fig. 8. Mycelium on wort agar.

Fig. 9. A spore which has germinated and produced a large lateral cell.

Fig. 10. Racquet mycelium with a large pyriform conidium.

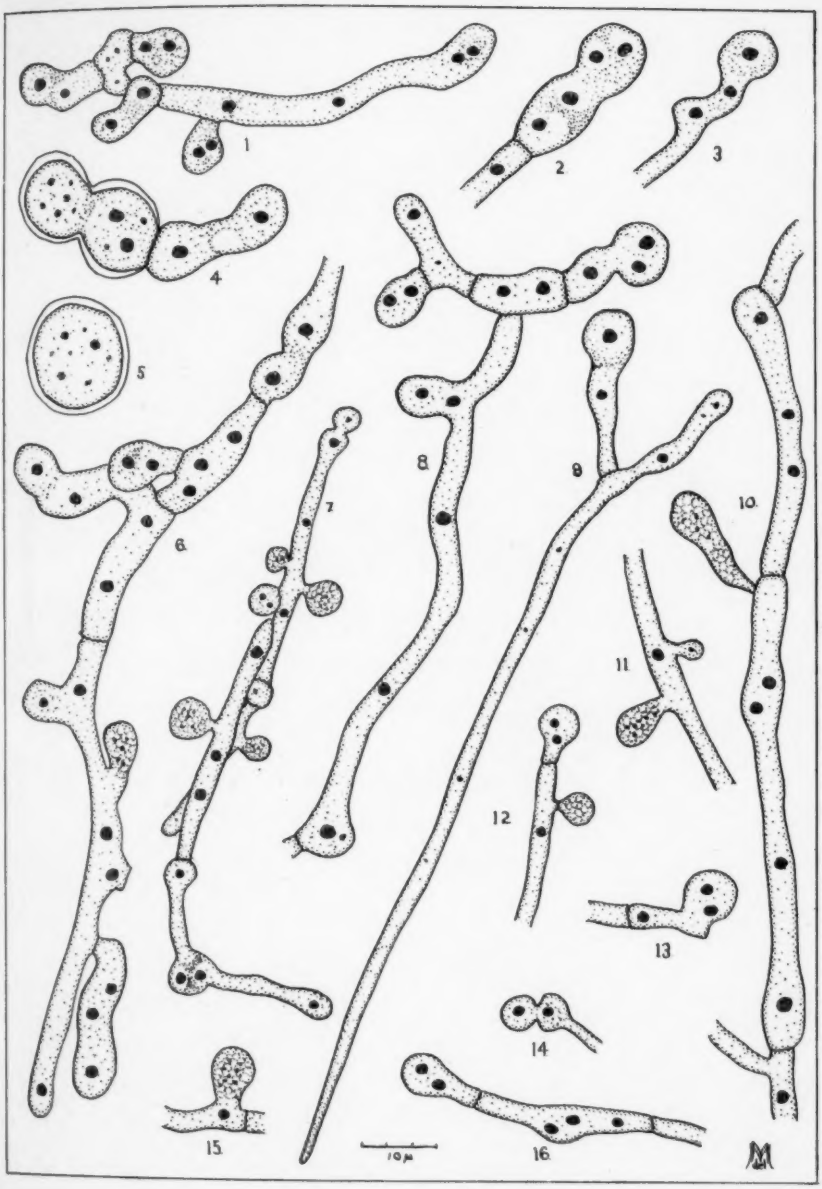
Fig. 11. Conidia on lactose agar.

Fig. 12. Terminal chlamydospore and lateral conidium or perhaps chlamydospore on beef extract plus blood serum agar.

Fig. 13. Lateral chlamydospore.

Figs. 14, 16. Cells of the above.

Fig. 15. Conidium on wort agar.



MOORE—ENDOMYCES CAPSULATUS

Explanation of Plate

PLATE 19

All drawings made with the aid of a camera lucida at × 1440.

Figs. 1–4. Germination of spores on 2 per cent bacto-peptone plus 5 per cent meat extract.

Fig. 5. Pyriform, sessile conidium on Richards' solution agar.

Fig. 6. Ascus.

Fig. 7. Hyphae showing round conidia and an intercalary chlamydospore.

Fig. 8. Mycelium with chlamydospores and conidia on Raulin's solution agar.

Fig. 9. Bit of young hypha with a large chlamydospore on Richards' solution agar.

Fig. 10. Terminal hypnospore and a lateral chlamydospore on Raulin's solution agar.

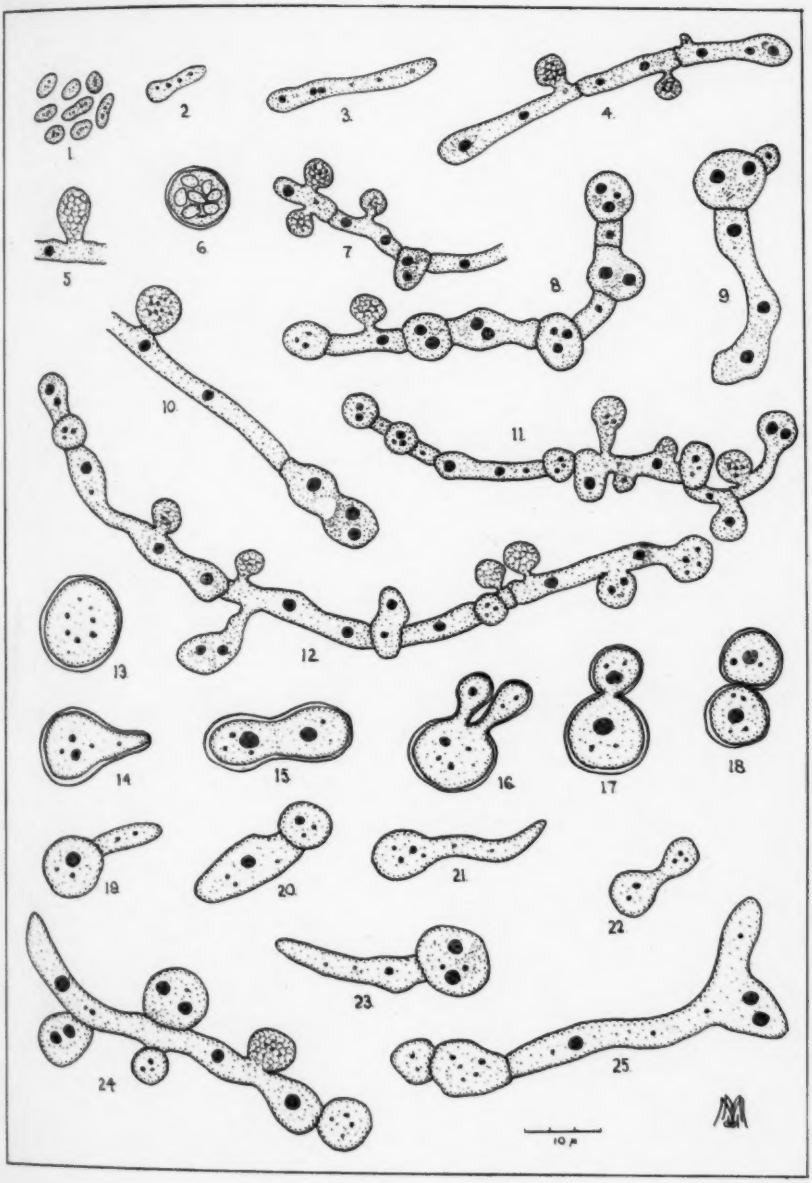
Fig. 11. Hypha with numerous pyriform and round conidia as well as chlamydospores.

Fig. 12. Hypha showing conidia, chlamydospores, and probably an ascogenous cell.

Figs. 13–25. Yeast-like cells and hyphae of the organism from the Austrian case on Sabouraud's agar.

Figs. 13–18. Showing budding.

Figs. 19–25. Formation of hyphae with conidia and chlamydospores.



MOORE—ENDOMYCES CAPSULATUS

EXPLANATION OF PLATE

PLATE 20

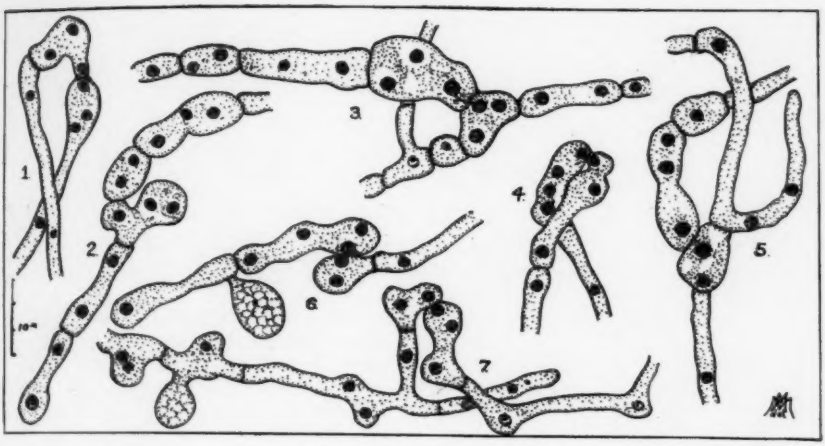
A

Forms of copulation drawn with the aid of a camera lucida at a magnification of × 1440.

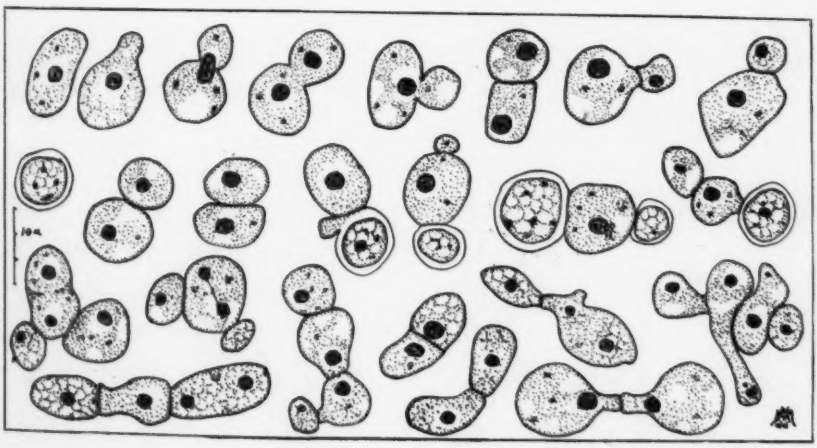
Fig. 1. Terminal copulation.
Fig. 2. Probably a form of copulation.
Fig. 3. Termino-lateral copulation.
Fig. 4. Same as fig. 1.
Fig. 5. Same as fig. 3.
Fig. 6. Lateral form of terminal copulation.
Fig. 7. Terminal copulation.

B

Cells observed on a medium which contained a high pH. Reversion to a yeast-like form × 1440.



A



B

MOORE—ENDOMYCES CAPSULATUS

EXPLANATION OF PLATE

PLATE 21

All drawings made at a magnification of × 1440 with the aid of a camera lucida.

Figs. 1–8. Yeast-like and budding cells on 2 per cent bacto-peptone (aqueous). Fresh isolation.

Figs. 9–11. Saccharomycetous cells developing mycelium in a hanging-drop culture of 2 per cent aqueous solution of bacto-peptone plus 5 per cent meat extract.

Figs. 12–13. Later stages of the above.

Fig. 14. Heterogamous copulation, premature stage on Sabouraud's agar.

Fig. 15. Heterogamous copulation seen at time of transfer of nuclear material, in hanging-drop of 2 per cent aqueous proteose peptone plus glycerine.

Fig. 16. Germinating spore on glycerine agar.

Fig. 17. Germinating spore developing hyphae on lactose agar.

Fig. 18. Developing ascus on Sabouraud's agar.

Fig. 19. Ascus showing 8 spores on nutrient agar.

Fig. 20. Racquet mycelium on lactose agar.

Fig. 21. Oidia-like cells formed in Sabouraud's broth.

Fig. 22. Ascus on potato-dextrose agar.

Fig. 23. Terminal hypnospore (chlamydospore) on glycerine agar.

Fig. 24. Large round chlamydospore with a thick wall on corn-meal agar.

Fig. 25. Section of a mycelium showing a thick-walled chlamydospore in a hanging-drop of 2 per cent bacto-peptone (aqueous).

Fig. 26. Mycelium with conidia on glycerine agar.

Fig. 27. Mycelium on eosine-methylene-blue agar.

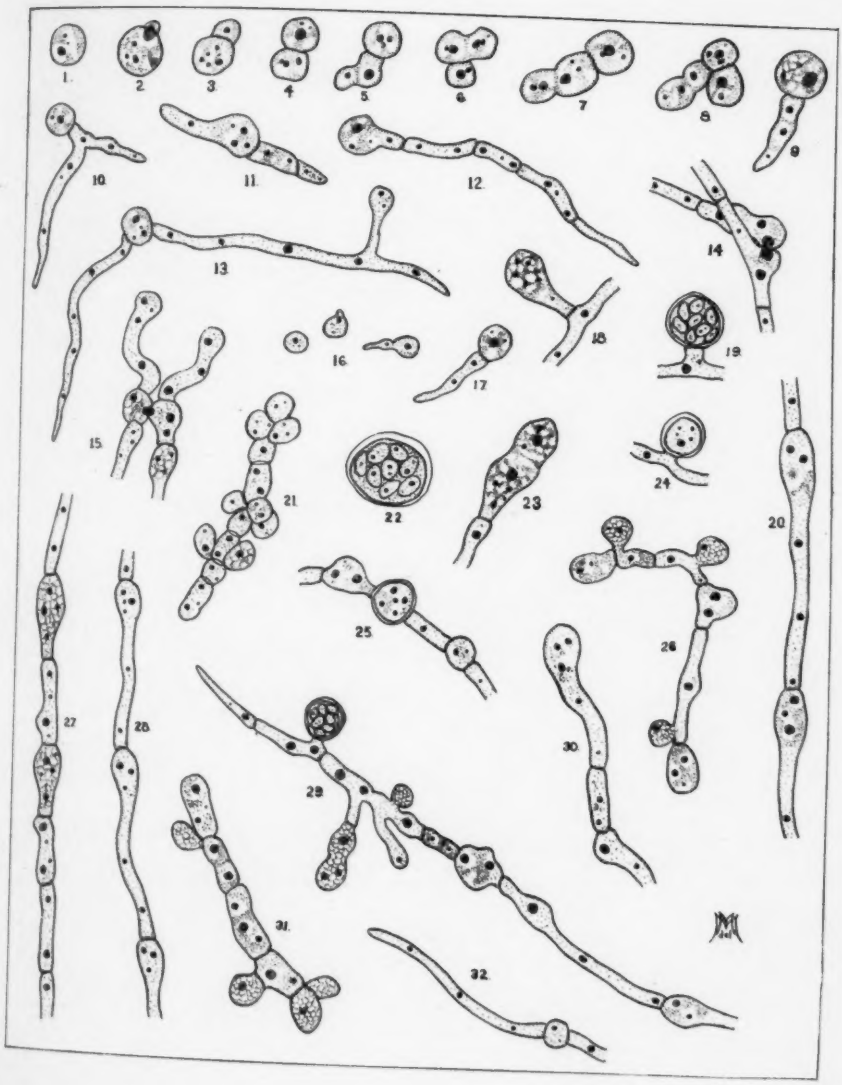
Fig. 28. Racquet mycelium on Sabouraud's agar.

Fig. 29. Mycelium showing racquet formation, conidia, hypnospores, and an 8-spored ascus, on glycerine agar.

Fig. 30. Terminal hypnospore on 2 per cent proteose peptone plus glycerine.

Fig. 31. Monilia-like growth in nutrient broth.

Fig. 32. Young hypha, 2 per cent bacto-peptone hanging-drop culture.



MOORE—ENDOMYCES CAPSULATUS

VAR. ISABELLINUS

EXPLANATION OF PLATE

PLATE 22

All drawings made with the aid of a camera lucida and high oil-immersion magnification. × 2300.

All cultures grown on glycerine agar.

Figs. 1–3. Germinating spores. Fixed with Hermann's and stained with iron haematoxylin.

Fig. 4. Young hypha prior to septum formation showing many nuclei, some dividing.

Fig. 5. Hypha with septa formed.

Figs. 6–7. Antheridia with dividing nuclei. Fixed with Benda's and stained with iron haematoxylin.

Fig. 8. Nucleus after division. Fixed with Hermann's and stained with iron haematoxylin.

Fig. 9. Ascogonial cell.

Fig. 10. Copulation of antheridium and ascogonium of same hypha by means of a copulating tube.

Fig. 11. Copulation form with the antheridium larger than the ascogonium.

Fig. 12. Projection of copulating tube.

Fig. 13. Ascogenous cells with beginning fusion. Fixed with Benda's and stained with iron haematoxylin.

Fig. 14. Copulation of lateral cells. Fixed with Hermann's and stained with iron haematoxylin.

Fig. 15. Beginning dissolution of intervening walls in tube.

Fig. 16. Nuclear fusion.

Fig. 17. Transfer of nucleus to ascogonial cell and dissolution of tube.

Fig. 18. Fertilized ascogonium with large fusion nucleus, fixed in Hermann's and stained with iodine green.

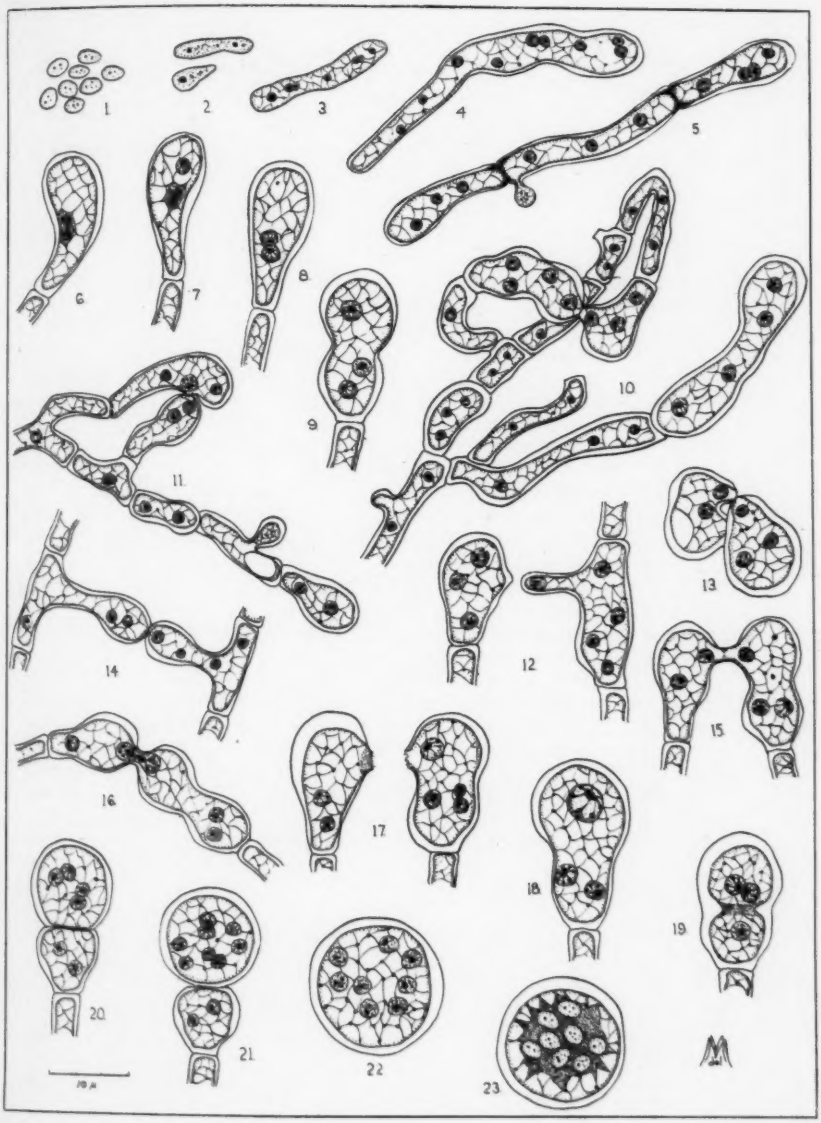
Fig. 19. Division of nucleus with beginning of formation of ascus, showing the abjunction of the special portion of the cell after syngamy. Fixed with Hermann's and stained with methylene blue.

Fig. 20. Synchronous division of nuclei. Fixed with Hermann's and stained with iron haematoxylin.

Fig. 21. Second synchronous division of nuclei with a rounding up of the future ascus.

Fig. 22. Maturation of ascus and ascospores.

Fig. 23. Matured spores in epiplasmic material.



MOORE—ENDOMYCES CAPSULATUS

EXPLANATION OF PLATE

PLATE 23

All drawings made with the aid of a camera lucida and high oil-immersion magnification. × 2300.

Figs. 1–18. Grown on glycerine agar.

Figs. 19–38. Living material.

Figs. 1–9. Represent development of an ascus parthenogenetically. Fixed with Hermann's and stained with iron haematoxylin.

Fig. 10. Conidium.

Fig. 11. Cross-section of a cell showing the reticulated network.

Fig. 12. Two divided nuclei in a terminal cell.

Fig. 13. A parthenogenetically formed ascus (terminal) with spores in the center and surrounded with epiplasm.

Fig. 14. Terminal cell showing vacuoles formed by reticulum, fixed with Hermann's and stained with iodine green.

Fig. 15. Vacuolar formation in cell fixed with Hermann's and stained with methylene blue.

Fig. 16. Metachromatic granules stained with methylene blue.

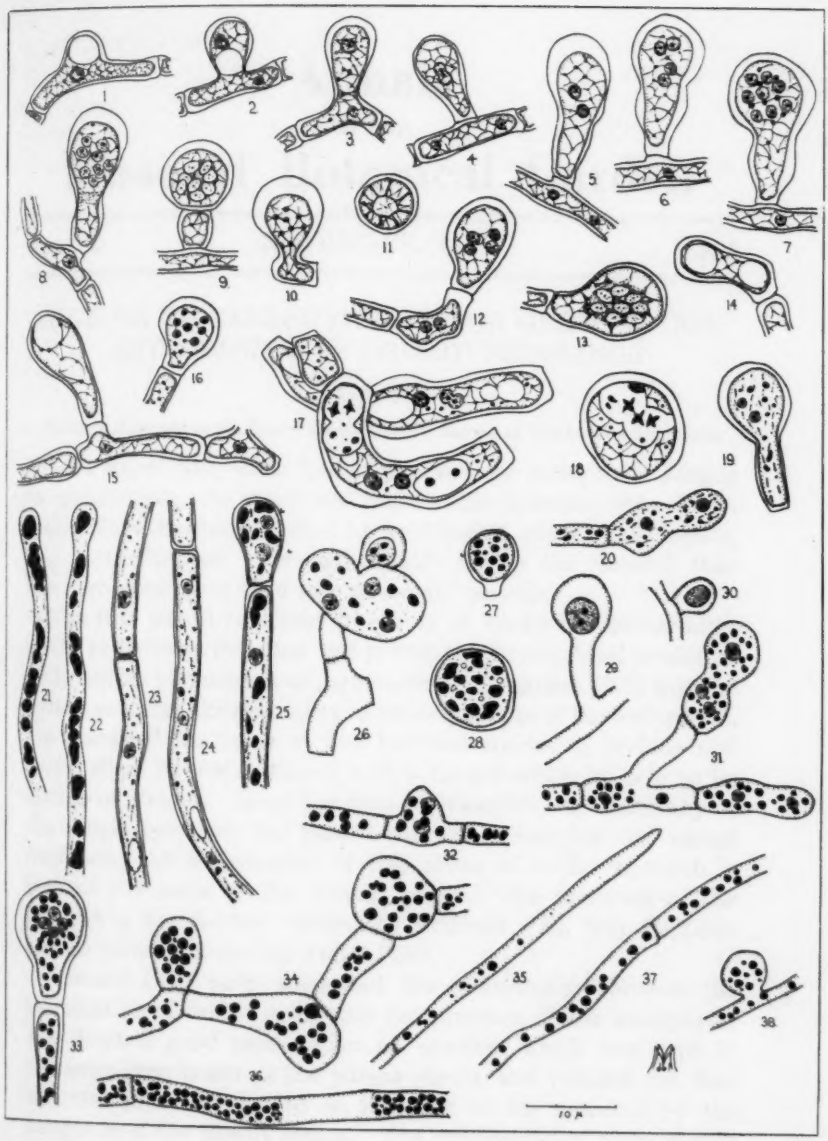
Figs. 17–18. Volutin in vacuoles.

Figs. 19–25, 27–28. Living cells stained and fixed with iodine potassium iodide, showing glycogen, lipoidal material and probable chondriosomes. The glycogen stains darkly (figs. 21–22, 25), the lipoidal substances are small, granular, and hyaline, and the chondriosomes are rod-like. Figs. 27–28 show young asci.

Figs. 26, 29–30. Living cells stained with neutral red, showing glycogen content.

Figs. 31–36. Living hyphae fixed with osmic acid, showing lipoidal or fat substances. Fig. 35, a young hypha, and fig. 36, an older hypha.

Figs. 37–38. Living material fixed with platinic chloride.



MOORE—ENDOMYCES CAPSULATUS